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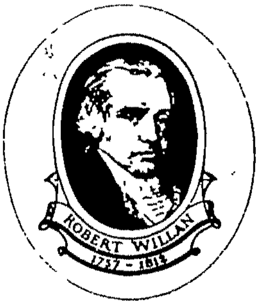

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# Contents

## No. 1: JULY 1986

### Laboratory and Clinical Investigations

Vehicle effects on percutaneous absorption: *in vivo* and *in vitro* comparisons with human skin 1
R.L.BRONAUGH AND T.J.FRANZ

Monoclonal antibodies against epidermal cell surface and dermal-epidermal junction: antigens which may be epidermal cell spreading factors 13
H.KATAYAMA, J.KINO, S.ITAMI, M.TSUTSUI, H.KOIZUMI, K.M.HALPRIN AND K.ADACHI

Lichen planus in relation to occupational and non-ocupational exposure to chemicals 23
C.LIDÉN

Nickel dermatitis: the reaction to oral nickel challenge 33
D.J.GAWKRODGER, S.W.COOK, G.S.FELL AND J.A.A.HUNTER

Electrical field stimulation of isolated primate sweat glands 39
G.I.VITALE, R.P.QUATRALE, P.J.GILES AND J.E.BIRNBAUM

Gianotti-Crosti syndrome: a study of 26 cases 49
A.TAIEB, PATRICE PLANTIN, P.DU PASQUIER, G.GUILLET AND J.MALEVILLE

Incontinentia pigmenti: eosinophil chemotactic activity of the crusted scales in the vesiculobullous stage 61
H.TAKEMATSU, T.TERUI, W.TORINUKI AND H.TAGAMI

Use of UV-A sunbeds for cosmetic tanning 67
B.L.DIFFEY

### Pharmacology and Treatment

Treatment of polymorphous light eruption with nicotinamide: a pilot study 77
R.NEUMANN, E.RAPPOLD AND HELGA POHL-MARKL

The surgical treatment of upper limb hyperhidrosis 81
P.S.MALONE, A.E.P.CAMERON AND J.A.RENNIE

### Case Reports

Concurrent multiple morphea and neonatal lupus erythematosus in an infant boy born to a mother with SLE 85
N.OHTAKI, C.MIYAMOTO, M.ORITA, M.KOYA AND M.MATSUO

Giant solitary trichoepitheliomas located in the perianal area: a report of three cases 91
F.M.TATNALL AND E.WILSON JONES

Microcystic adnexal carcinoma 101
F.RONGIOLETTI, E.GROSSHANS AND A.REBORA

Atrophia maculosa varioliformis cutis 105
V.J.MARKS AND O.F.MILLER

Three cases of eccrine porocarcinoma 111
L.PUTTICK, P.INCE AND J.S.COMAISH

### Correspondence

Subcorneal pustular dermatosis followed by pyoderma gangrenosum 117
V.A.VENNING AND T.J.RYAN

**Book Reviews** 119

**News and Notices** 122

## No. 2: AUGUST 1986

### Invited Editorial

The electron microscope and the antibody: trends in the diagnosis of epidermolysis bullosa 123
CHRISTINE J.SKERROW

Reply 254
J.FERGUSON AND B.E.JOHNSON
Arterial lesions of the lower lip 254
A.FRUMKIN
Reply 255
R.J.MARSHALL AND B.J.LEPPARD

**Book Reviews** 256

**News and Notices** 258

## No. 3: SEPTEMBER 1986

**Invited Editorial**

Hiradenitis suppurativa: an androgen-dependent disorder 259
F.J.G.EBLING

**Laboratory and Clinical Investigations**

A double-blind controlled cross-over trial of cyproterone acetate in females with hidradenitis suppurativa 263
P.S.MORTIMER, R.P.R.DAWBER, MARY A.GALES AND R.A.MOORE
Control of hidradenitis suppurativa in women using combined antiandrogen (cyproterone acetate) and oestrogen therapy 269
R.S.SAWERS, VALERIE A.RANDALL AND F.J G EBLING
Photosensitivity due to retinoids: clinical and laboratory studies 275
J.FERGUSON AND B.E.JOHNSON
Potentiation of the generation of reactive oxidants by human phagocytes during exposure to benoxaprofen and ultraviolet radiation *in vitro* 285
R.ANDERSON AND H.A.EFTYCHIS
Ionophore A23187 stimulates arachidonic acid release and cyclic GMP accumulation in pig epidermis 297
N.KATO, H.SUYA, T.AOYAGI, J.R.TAYLOR AND K.M.HALPRIN
Immunopathological and ultrastructural findings in human allergic and irritant contact dermatitis 305
C.M.WILLIS, E.YOUNG, D.R.BRANDON AND J.D.WILKINSON
Fine needle aspiration cytology in dermatology: a clinicopathological appraisal 317
D.N.SLATER AND G.REILLY
A study of HLA antigen association in localized and generalized granuloma annulare 329
R.FRIEDMAN-BIRNBAUM, O.GIDEONI, R.BERGMAN AND S.POLLACK
Animal models for percutaneous penetration. assessing contamination of the experimental system 335
C.L.GUMMER AND H.MAIBACH
Seborrhoeic eczema—a disease entity or a clinical variant of atopic eczema? 341
P.PODMORE, D.BURROWS, D.J.EEDY AND C.F.STANFORD
Immunogold labelling of epidermal Langerhans cells on tissue sections of normal human skin 351
G.DE PANFILIS, G.C.MANARA AND C.FERRARI

**Case Reports**

Fixed drug eruption due to paracetamol 357
R.H.MEYRICK THOMAS AND D.D.MUNRO
Periungual and subungual arteriovenous tumours 361
S.M.BURGE, R.BARAN, R.P.R.DAWBER AND J.L.VERRET
Thalidomide in the treatment of neuro-Behçet's syndrome 367
C.G.RAMSELAAR, R.M.BOONE AND H.C.KLUIN-NELEMANS
Cholinergic dermographism 371
S.C MAYOU, A.KOBZA BLACK, R.A.J.EADY AND M.W.GREAVES
Occupational high-pressure injection injury 379
J.C.MACAULAY

**Correspondence**

Poikiloderma vasculare atrophicans granulomatosa 383
P.C.M.VAN DE KERKHOF, B.E.W.L.VAN HUYSTEE, U.J G.M.VAN HAELST AND M.J.J.T.BOGMAN
Acne and unemployment 386
W.J CUNLIFFE
Lasers and psoriasis 386
P.V.HARRISON

**Book Reviews** 387

**News and Notices** 388

## No. 4: OCTOBER 1986

### Invited Editorial

Do epidermotropic T cells exist in normal human skin? A re-evaluation of the SALT hypothesis 389
S.M.BREATHNACH

### Clinical and Laboratory Investigations

Scl 70 antibody—a specific marker of systemic sclerosis 393
MARIA JARZABEK-CHORZELSKA, MARIA BLASZCZYK, STEFANIA JABLONSKA, T.CHORZELSKI, V.KUMAR AND E.H.BEUTNER

Degradation of porcine dermal connective tissue by collagenase and hyaluronidase 403
K.FRIEDMAN, S.V.POLLACK, T.MANNING AND S.R.PINNELL

Immunolocalization of collagenase and tissue inhibitor of metalloproteinases (TIMP) in hypertrophic scar tissue 409
ROSALIND M.HEMBRY AND H.P.EHRLICH

An open study of vitamin $D_3$ treatment in psoriasis vulgaris 421
S.MORIMOTO, K.YOSHIKAWA, T.KOZUKA, Y.KITANO, S.IMANAKA, K FUKUO, E.KOH AND Y.KUMAHARA

Successful treatment of psoriasis with topical application of active vitamin $D_3$ analogue, $1\alpha$,24-dihydroxycholecalciferol 431
T.KATO, M.ROKUGO, T.TERUI AND H TAGAMI

Circulating antibodies and antigenic cross-reactivity in *Hendersonula toruloidea* and *Scytalidium hyalinum* infections 435
M.K.MOORE AND R.J.HAY

HLA antigens and Langerhans cell density in contact dermatitis 447
S.I.WHITE, P.S FRIEDMANN AND A.STRATTON

A quantitative study of the effect of topical indomethacin on cutaneous erythema induced by UVB and UVC radiation 453
P.M.FARR AND B.L.DIFFEY

Behavioural treatment of scratching in patients with atopic dermatitis 467
L MELIN, T.FREDERIKSEN, P NOREN AND B G.SWEBILIUS

Effect of percutaneous absorption of hydrocortisone on adrenocortical responsiveness in infants with severe skin disease 475
M.TURPEINEN, O.P.SALO AND S.LEISTI

Challenge reactions in atopic dermatitis after percutaneous entry of mite antigen 485
A.GONDO, N.SAEKI AND Y.TOKUDA

### Case Reports

Secondary syphilis mimicking Sweet's syndrome 495
H.F.JORDAAN AND J.CILLIERS

Giant orf in a normal individual 497
J V.S.PETHER, C.J.W.GUERRIER, S.M.JONES, A.E.ADAM AND W.N.KINGSBURY

Blisters over burn scars in a child 501
R.PEDRAGOSA, S.SERRANO, J.CAROL-MURILLO, J.V.HERNANDEZ AND J.VIDAL

### Obituary

Hermann Pinkus 507

### Correspondence

Expression of T6 antigen on the epidermal keratinocytes in various dermatoses (I) 511
M.DRIJKONINGEN, C.DE WOLF-PEETERS AND V.DEMSET

Expression of T6 antigen on the epidermal keratinocytes in various dermatoses (II) 512
C.PINCELLI, G.GIROLOMONI AND L.BENASSI

Reply 513
R.WILLEMZE

Natural killer cells and lymphomatoid granulomatosis 514
F.RONGIOLETTI, MARIA GRAZIA BERNENGO, F.CROVATO AND A.REBORA

Persistent phototoxicity after benoxaprofen 515
I.B.SNEDDON

# Contents

## No. 1: JULY 1986

### Laboratory and Clinical Investigations

Vehicle effects on percutaneous absorption: *in vivo* and *in vitro* comparisons with human skin 1
R.L.BRONAUGH AND T.J.FRANZ
Monoclonal antibodies against epidermal cell surface and dermal-epidermal junction: antigens which may be epidermal cell spreading factors 13
H.KATAYAMA, J.KINO, S.ITAMI, M.TSUTSUI, H.KOIZUMI, K.M.HALPRIN AND K.ADACHI
Lichen planus in relation to occupational and non-ocupational exposure to chemicals 23
C.LIDÉN
Nickel dermatitis: the reaction to oral nickel challenge 33
D.J.GAWKRODGER, S.W.COOK, G.S.FELL AND J.A.A.HUNTER
Electrical field stimulation of isolated primate sweat glands 39
G.I.VITALE, R.P.QUATRALE, P.J.GILES AND J.E.BIRNBAUM
Gianotti-Crosti syndrome: a study of 26 cases 49
A.TAIEB, PATRICE PLANTIN, P.DU PASQUIER, G.GUILLET AND J.MALEVILLE
Incontinentia pigmenti: eosinophil chemotactic activity of the crusted scales in the vesiculobullous stage 61
H.TAKEMATSU, T.TERUI, W.TORINUKI AND H.TAGAMI
Use of UV-A sunbeds for cosmetic tanning 67
B.L.DIFFEY

### Pharmacology and Treatment

Treatment of polymorphous light eruption with nicotinamide: a pilot study 77
R.NEUMANN, E.RAPPOLD AND HELGA POHL-MARKL
The surgical treatment of upper limb hyperhidrosis 81
P.S.MALONE, A.E.P.CAMERON AND J.A.RENNIE

### Case Reports

Concurrent multiple morphea and neonatal lupus erythematosus in an infant boy born to a mother with SLE 85
N.OHTAKI, C.MIYAMOTO, M.ORITA, M.KOYA AND M.MATSUO
Giant solitary trichoepitheliomas located in the perianal area: a report of three cases 91
F.M.TATNALL AND E.WILSON JONES
Microcystic adnexal carcinoma 101
F.RONGIOLETTI, E.GROSSHANS AND A.REBORA
Atrophia maculosa varioliformis cutis 105
V.J.MARKS AND O.F.MILLER
Three cases of eccrine porocarcinoma 111
L.PUTTICK, P.INCE AND J.S.COMAISH

### Correspondence

Subcorneal pustular dermatosis followed by pyoderma gangrenosum 117
V.A.VENNING AND T.J.RYAN

**Book Reviews** 119

**News and Notices** 122

## No. 2: AUGUST 1986

### Invited Editorial

The electron microscope and the antibody: trends in the diagnosis of epidermolysis bullosa 123
CHRISTINE J.SKERROW

## Laboratory and Clinical Investigations

Identification of an epidermal basement membrane defect in recessive forms of dystrophic epidermolysis bullosa by LH 7:2 monoclonal antibody: use in diagnosis 125
A.H.M.HEAGERTY, A.R.KENNEDY, I.M.LEIGH, P.PURKIS AND R A.J.EADY
Secretion of plasminogen activator inhibitor by cultured human epidermal cells 133
K.HASHIMOTO, K.NISHIOKA, I.KATAYAMA AND K.YOSHIKAWA
Contraction of collagen lattices by fibroblasts from patients and animals with heritable disorders of connective tissue 139
P.DELVOYE, CORNELIA MAUCH, T.KRIEG AND C.M.LAPIERE
Elevated thymosin alpha-1 levels in Brazilian pemphigus foliaceus 147
J.T.ROSCOE, P.H.NAYLOR, L.A.DIAZ, R.S.LABIB, H.P.PATEL, A.L.GOLDSTEIN, S.A.P.SAMPAIO AND G.J.ANHALT
An assessment of the diagnostic value of the monoclonal antibodies Leu 8, OKT9, OKT10 and Ki67 in cutaneous lymphocytic infiltrates 151
MARLYN L.TURBITT AND RONA M.MACKIE
Leukapheresis in the treatment of cutaneous T-cell lymphomas 159
S.V.BELTER, J.KNOP, K.BRUSKE AND C.SORG
Patterns of dermatology referrals in a general hospital 167
N.HARDWICK AND NORMA SAXE
Brachioradial pruritus: a tropical dermopathy 177
PATRICIA J.WALCYK AND D.J.ELPERN
Elastase, a marker for neutrophils in skin infiltrates 181
A.M.LAMMERS, P.C.M.VAN DE KERKHOF, J.SCHALWIJK AND P.D.MIER
Immunohistochemical study of lysozyme in lupus miliaris disseminatus faciei 187
K.MIHARA AND M.ISODA
Hyper-induction of skin ornithine decarboxylase activity in psoriatic patients 193
M.BOUCLIER, P.ELBAZE, G.MILANO, B.SHROOT AND J-P.ORTONNE
The autoimmune background in lichen planus 199
D.SHUTTLEWORTH, R.A.C.GRAHAM-BROWN AND A C.CAMPBELL
Plasminogen activator secreted by cultured human melanocytes 205
K.HASHIMOTO, T.HORIKOSHI, K.NISHIOKA, K.YOSHIKAWA AND D.M.CARTER

## Pharmacology and Treatment

Treatment of chronic discoid lupus erythematosus with an oral gold compound (auranofin) 211
K.DALZIEL, G.GOING, P.H.CARTWRIGHT, R.MARKS, G.W.BEVERIDGE AND N.R.ROWELL
Itraconazole therapy in pityriasis versicolor 217
A.DEL PALACIO HERNANZ, J.FRIAS-INIESTA, O.GONZALEZ-VALLE, M.BORGERS, J.VAN CUTSEM AND G.CAUWENBERGH
Oral spironolactone: an effective treatment for acne vulgaris in women 227
M.F.MUHLEMANN, G.D.CARTER, J.J.CREAM AND P.WISE
The measurement of the stratum corneum reservoir. a simple method to predict the influence of vehicles on *in vivo* percutaneous absorption 233
D.DUPUIS, A.ROUGIER, R.ROGUET AND CLAIRE LOTTE

## Case Reports

Metastasizing atrial myxoma: a case with multiple subcutaneous tumours 239
N.OKADA, T.YAMAMURA, Y.KITANO, T.NAKAMURA, H.KAMIDO, Y.MATSUZAWA, S.KATAYAMA AND K.OHARA
A syndrome of keratosis palmo-plantaris congenita, pes planus, onychogryphosis, periodontosis, arachnodactyly and a peculiar acro-osteolysis 243
J.M.PULIYEL AND K.S.SRIDHARAN IYER

## Correspondence

Degos' disease 249
JULIA A.NEWTON AND M.M.BLACK
Cyclosporin A and psoriasis 249
B.E.MONK
Elevated serum amylase in toxic epidermal necrolysis 250
H.TAGAMI AND K.IWATSUKI
Reply 250
F.M.TATNALL, H.J.DODD AND I.SARKANY
Anthralin inflammation: lack of effect of topical thiocarbamide drugs 251
Y.PARAMSOTHY, C.M.LAWRENCE AND S.SHUSTER
Haematological consequences of methotrexate therapy 253
H.BAKER
Amiodarone-induced cutaneous photosensitivity 253
J.BOYLE

Iontophoresis 516
J.LOW
Arachidonic acid metabolism by polymorphonuclear leukocytes in psoriasis 517
T.RUZICKA
Reply 518
P.D.L.MAURICE AND B.R.ALLEN

**Book Reviews** 519

**News and Notices** 520

## No. 5: NOVEMBER 1986

A relative thrombocytosis and elevated mean platelet volume are features of gravitational disease 521
M.J.D.GOODFIELD
Support and stimulation of epidermal cell outgrowth from porcine skin explants by platelet factors 529
PATRICIA A.HEBDA, STEPHANIE P.ALSTADT, W.T.HILEMAN AND W.H.EAGLSTEIN
*In vivo* induction of Ia antigens on rat keratinocytes by γ-interferon 543
A.SCHEYNIUS, C.JOHANSSON AND P.H.VAN DER MEIDE
The *in vitro* antimicrobial effect of azelaic acid 551
J.P.LEEMING, K.T.HOLLAND AND R.A.BOJAR
Demonstration of a unique viral structure. the molluscum viral colony sac 557
W.B.SHELLEY AND VERONIKA BURMEISTER
Excision of skin tumours without wound closure 563
C.M.LAWRENCE, J.S COMAISH AND M.G.C.DAHL
No association between malignant melanoma and acne or psoriasis: results from the Western Canada Melanoma Study 573
J.M.ELWOOD, R.P.GALLAGHER AND P.J.STAPLETON
Low-dose etretinate in the maintenance of remission of palmoplantar pustular psoriasis 577
S.I.WHITE, LINDA PUTTICK AND JANET M.MARKS

**Society Proceedings**

Proceedings of the 236th Meeting of the Netherlands Society for Dermatology and Venereology 583

**Case Reports**

Bazex paraneoplastic acrokeratosis: a case report and response to Tigason 595
J.M.WISHART
Acute HTLV III infection associated with exanthema, diagnosed by seroconversion 601
GUNHILD R.LANGE WANTZIN, B.ØRSKOV LINDHARDT, KAARE WEISMANN AND KAY ULRICH
Skin lesions from hypersensitivity to cold during chronic myelomonocytic leukaemia 607
B.DRENO, P.GANDON, B.BUREAU, N.MILPIED AND H.BARRIÈRE
Atypical angiomatosis in polyarteritis nodosa 611
R.F.HORAN, S.R.TAHAN, R.J.MARGOLIS AND H.A.HAYNES
Granulomatous cheilitis and a positive Kveim test 619
S.A.SHEHADE AND I.S.FOULDS
A case of multicentric reticulohistiocytosis: uncommon clinical signs and a report of T-cell marker characteristics 623
C.A.GREEN, D.J.WALKER AND A.J.MALCOLM
Generalized pustular psoriasis precipitated by trazodone in the treatment of depression 629
J.H.BARTH AND H.BAKER
Papular and nodular mucinosis associated with systemic lupus erythematosus 631
F.RONGIOLETTI AND A.REBORA

**Correspondence**

First blast of the trumpet against the monstrous regiment of scientists (with apologies to John Knox) 637
A.W.MCKENZIE
Acro-osteolysis and the nail unit 638
R.K.SCHER
Methotrexate for intractable benign familial chronic pemphigus 638
G.M.FAIRRIS, J.E WHITE, B.J.LEPPARD AND P.G.GOODWIN
Wallace's Line in serum sickness and Kawasaki disease 640
J.D.BERNHARD, A.R.RHODES AND J.W.MELSKI

**Book Reviews** 641

**News and Notices** 645

## No. 6: DECEMBER 1986

**Comment**

Nomenclature of non-neoplastic vulval conditions 647
C.M.RIDLEY

**Clinical and Laboratory Investigations**

Spontaneous remission of solar keratoses: the case for conservative management 649
R.MARKS, P.FOLEY, G.GOODMAN, BRIDGET H.HAGE AND T.S.SELWOOD
Pulmonary function in patients on long-term, low-dose methotrexate 657
T.J.PHILLIPS, P.J.WALLIS, D.H.JONES AND H.BAKER
The effect of altering area of application and dose per unit area on sensitization by DNCB 663
S.I.WHITE, P.S.FRIEDMANN, CELIA MOSS AND JUDY M.SIMPSON
Langerhans cells in human warts 669
YVETTE CHARDONNET, JACQUELINE VIAC AND J.THIVOLET
The effect of thalidomide on arachidonic acid metabolism in human polymorphonuclear leukocytes and platelets 677
P.D.L.MAURICE, A S.J.BARKLEY AND B.R.ALLEN
Human papillomavirus infections in a group of renal transplant recipients 681
R.RUDLINGER, I.W.SMITH, M.H.BUNNEY AND J.A.A.HUNTER

**Case Reports**

Erythroderma associated with mixed lymphocyte-endothelial cell interaction and *Staphylococcus aureus* infection 693
MADALENE C.Y.HENG, S.G.KLOSS AND D.G.CHASE
Longitudinal melanonychia with spreading pigmentation in Laugier-Hunziker syndrome: a report of two cases 707
R BARAN AND H.BARRIÈRE
Congenital smooth muscle hamartoma: a case report 711
BRENDA J.BERBERIAN AND J.W.BURNETT
Perineural xanthoma 715
H.NAKAYAMA, M.MIHARA AND S.SHIMAO
Release of prostaglandin $D_2$ and histamine in a case of localized heat urticaria, and effect of treatments 721
O.KORO, J.S.DOVER, D.M.FRANCIS, A.KOBZA BLACK, R.W.KELLY, R.M.BARR AND M.W.GREAVES
Oesophageal lichen planus 729
R.A.SHEEHAN-DARE, J.A.COTTERILL AND A.V.SIMMONS
Recurrent angioedema: familial and oestrogen-induced 731
R.P.WARIN, W.J.CUNLIFFE, M.W.GREAVES AND T.B.WALLINGTON
Acropustulosis of infancy 735
JULIA A.NEWTON, JENNIFER SALISBURY, A.MARSDEN AND D.H.MCGIBBON

**Obituary**

Claude Howard Whittle 741

**Correspondence**

Lichen sclerosus et atrophicus in non-identical female twins 743
N.H.COX, J.N.S MITCHELL AND W.N.MORLEY
Gamma-glutamyl transpeptidase as a marker for distinguishing malignant epithelial tumours 743
S T HUSSAIN
Influence of containers on the quantities of topical preparations used 743
C.D.EVANS AND J.WIGHTMAN
Lasers, dermal fibroblasts and psoriasis 744
P.SAIAG, B.COULOMB, C.M.E.ROWLAND-PAYNE, L.DUBERTRET AND R.TOURAINE
Cyclosporin A treatment of severe psoriasis 745
JANET M.MARKS

**Book Reviews** 747

**News and Notices** 750

**Index** 751

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**References:** 1 Data on file, Dermal Laboratories Ltd. 2 British National Formulary Number 8 (1984) British Medical Association and The Pharmaceutical Society.

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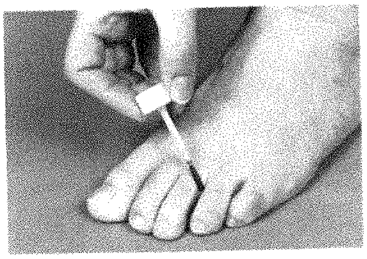

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*Reference*

[1] Krebs HA, Wiggins D, Stubbs M, Sols A, Bedoya F. Studies on the mechanism of the anti-fungal action of benzoate. Biochem J 1983; **214**: 657-63

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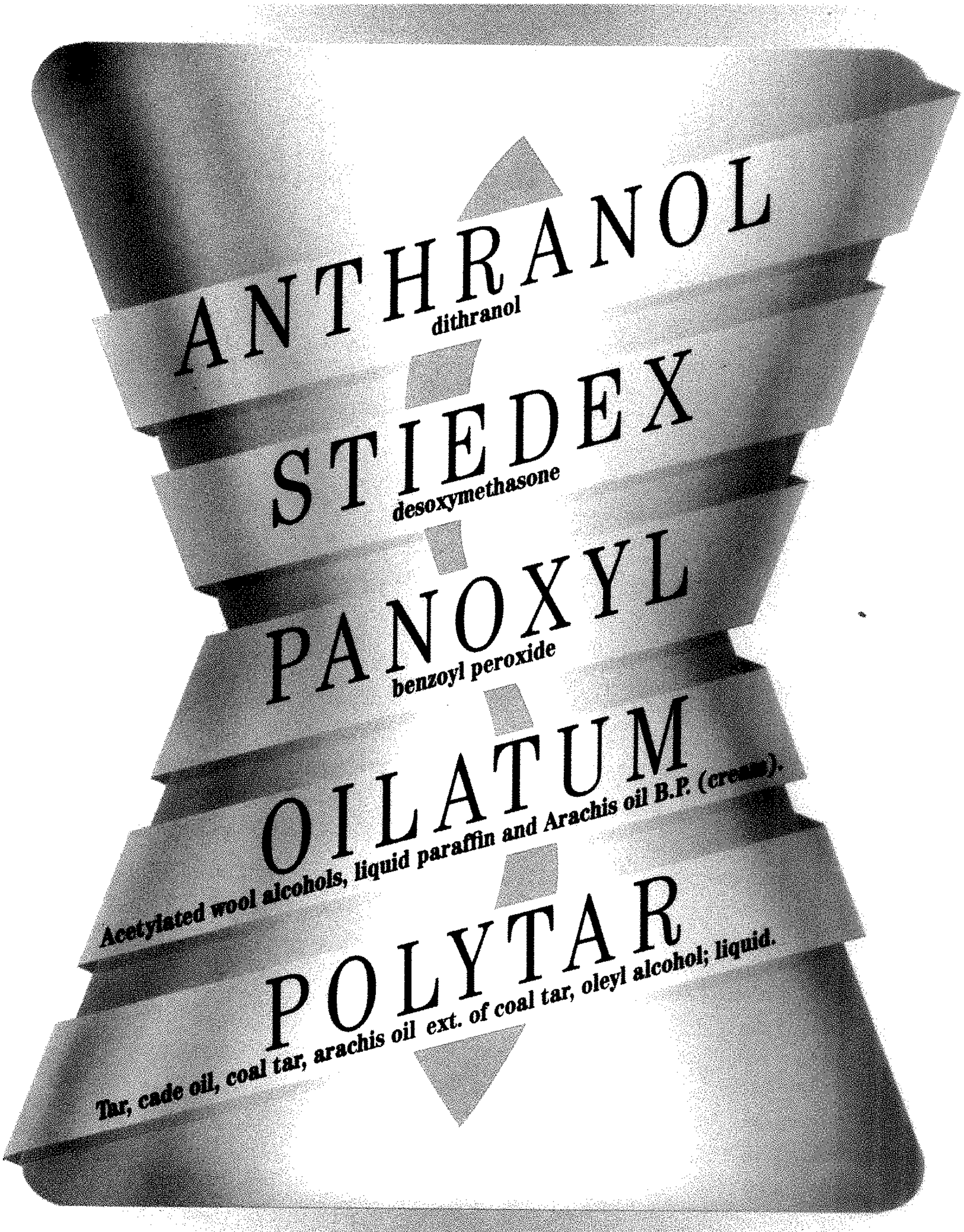
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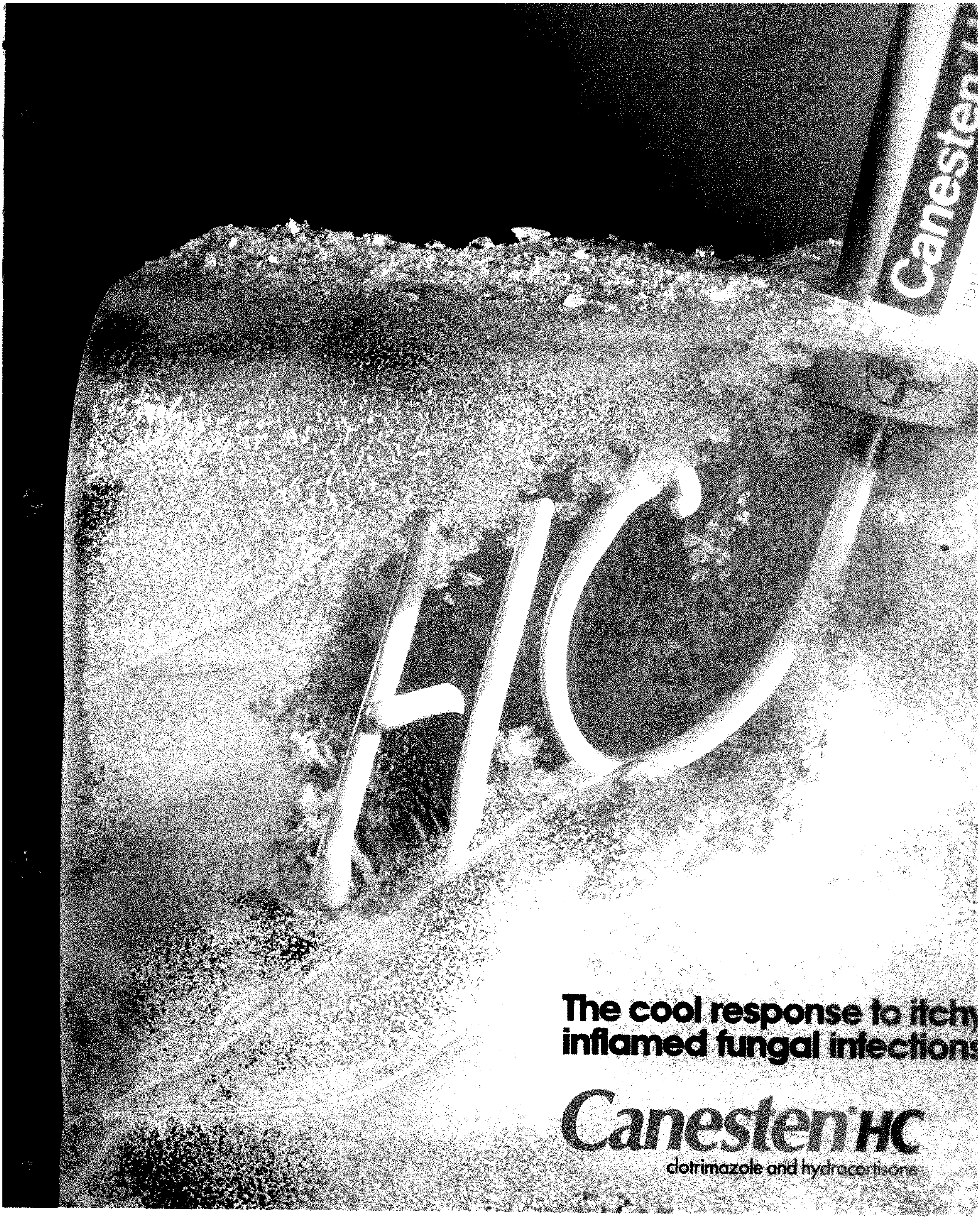
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1983. 53 figures, 8 color plates. XIX, 149 pages.
Hard cover DM 180,–. ISBN 3-540-90792-0

## Dermatopathology

Editor: C. L. Berrry, London

1985. 94 figures. IX, 286 pages.
(Current Topics in Pathology, Volume 74)
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**Contents:** Melanoma and Other Melanocytic Skin Lesions. – Cutaneous Vasculitis. – Genetic and Developmental Aspects of Pathological Pigmentation Patterns. – Changes in Epidermal Cell Proliferation in Proliferative Skin Diseases. – Mycosis fungoides. – Bullous Dermatoses.

J. H. S. Pettit, Kuala Lumpur; L. C. Parish, Philadelphia

## Manual of Tropical Dermatology

1984. 119 figures, 12 color plates. XVIII, 260 pages.
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## Lymphoproliferative Diseases of the Skin

Editors: M. Goos, E. Christophers, University of Kiel

1982. 149 figures, 82 tables. XV, 296 tables.
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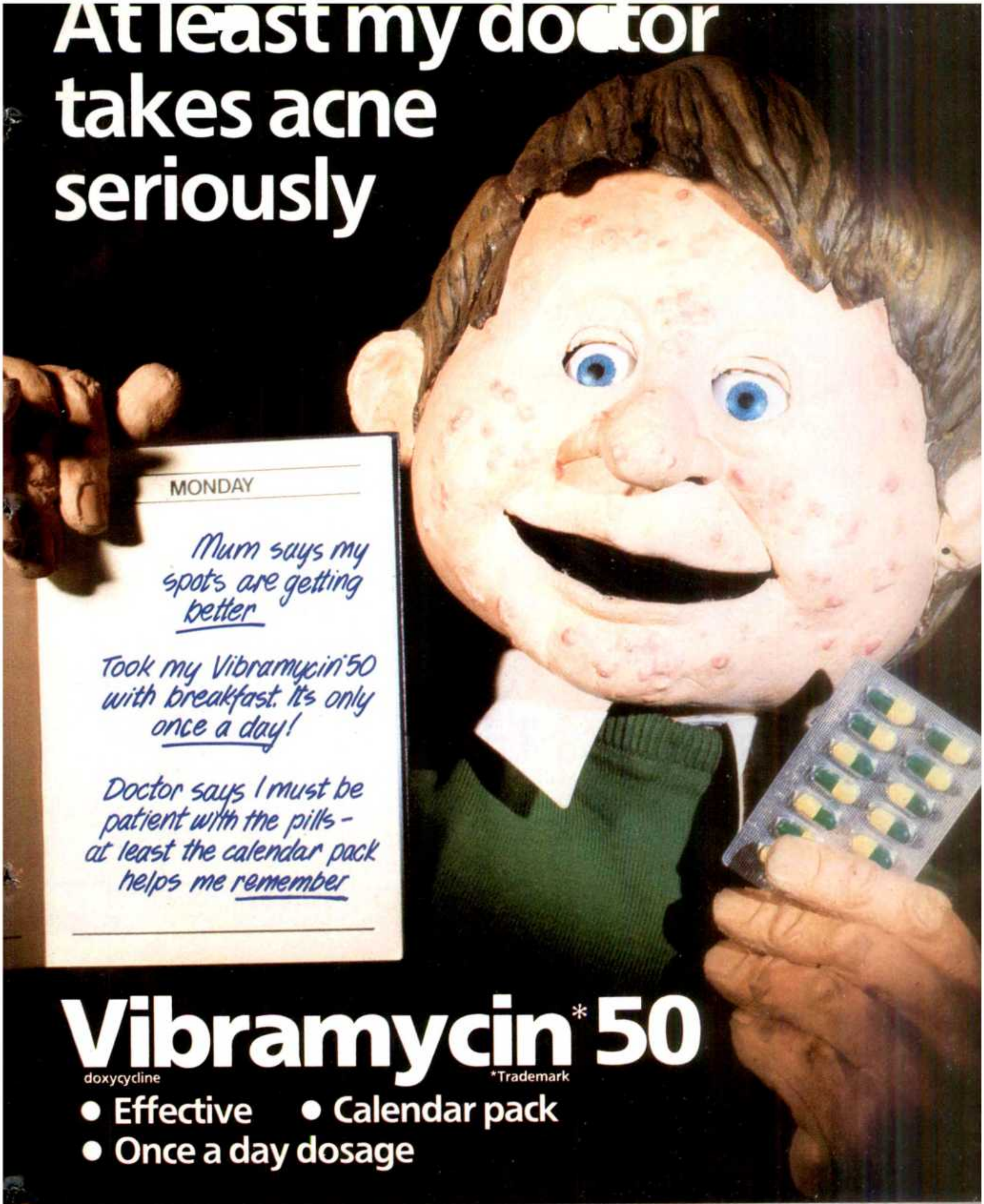
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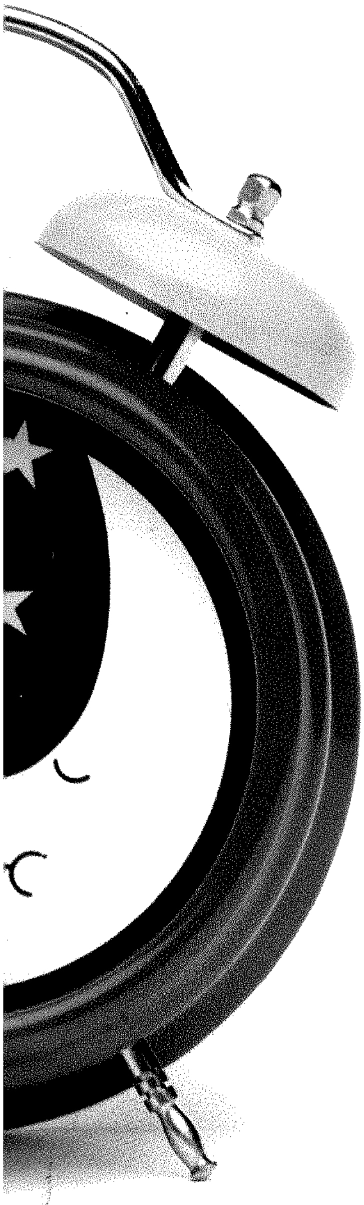

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*British Journal of Dermatology* (1986) **115**, 1–11.

***Laboratory and Clinical Investigations***

# Vehicle effects on percutaneous absorption: *in vivo* and *in vitro* comparisons with human skin

R.L.BRONAUGH AND T.J.FRANZ*

Division of Toxicology, Food and Drug Administration, Washington, D.C. and *Division of Dermatology, University of Washington School of Medicine, Seattle, Washington, U.S.A.

Accepted for publication 17 December 1985

## SUMMARY

The percutaneous absorption of benzoic acid, caffeine and testosterone through human skin was measured by using *in vivo* and *in vitro* techniques. The compounds were applied to the skin in solution in three vehicles: petrolatum, ethylene glycol gel and water gel. Because benzoic acid was ionized at the neutral pH of the gels, these data were difficult to interpret and are not reported. The stratum corneum/vehicle partition coefficients ($K_m$) and percent saturation of the vehicles with substrate were determined to aid in the interpretation of the absorption results. In the *in vitro* studies, the permeability constants determined for the compounds in each vehicle correlated with either the $K_m$ value or the percent saturation of the vehicle. Caffeine penetrated most readily from a petrolatum vehicle, and the greatest testosterone absorption was from a water gel. Permeation was also expressed in terms of the percentage of the applied dose absorbed. Reasonable agreement was obtained between the *in vivo* and *in vitro* values. Although no significant differences occurred between most values compared, there was a trend toward lower penetration in the *in vitro* system.

The assessment of percutaneous absorption of chemicals has become of increasing interest both from a pharmacological and toxicological standpoint. Comparisons of absorption through human and animal skin (Bartek, LaBudde & Maibach, 1972; Bronaugh, Stewart & Congdon, 1982) have shown that human skin is unique and should be used for the most meaningful results.

Because the sensitivity of radioisotopes is often required for quantitation of absorption, and because of the potential toxicity of certain test compounds, human *in vivo* studies are not always feasible. On the other hand, *in vitro* studies are safe, convenient and possibly more accurate than *in vivo* studies, since sampling of absorbed material can be made directly below the surface of the skin in diffusion cells (Kligman, 1983).

A number of comparative studies have demonstrated a similarity between *in vivo* and *in vitro*

Correspondence: Robert L.Bronaugh, PhD.

absorption, but only a few investigators have utilized human skin. Burch and Winsor (1944) found that trans-epidermal water loss through excised human skin was identical to that measured in living subjects. In several reports, Franz (1975, 1978) compared the *in vitro* absorption of 12 organic compounds (in an acetone vehicle) with the results of the original *in vivo* study by Feldmann and Maibach (1970). Good agreement between the studies was obtained and the few apparent discrepancies in absorption values could be explained by experimental differences.

Because of the small number of *in vivo* and *in vitro* absorption comparisons with human skin, the present study was undertaken. Unlike previous studies, compounds were applied to the skin in different vehicles to assess their effect on skin permeation. The methodology used in the two laboratories was closely co-ordinated. Stratum corneum/vehicle partition coefficients and compound solubility in vehicles were measured to aid in the interpretation of results. Compounds were selected that were expected to be absorbed readily on the basis of their chemical properties and previous absorption studies (Feldmann & Maibach, 1970; Franz, 1975, 1978). Good absorption was a requirement so that blood levels could be detected following topical application. This data will be published elsewhere.

## METHODS

### *Test formulations*

Radiolabelled compounds were used throughout the study to facilitate quantitation of absorbed material. In some cases unlabelled material was added to increase the dose of test chemical applied to the skin. [$^{14}$C]Benzoic acid (specific activity 29·4 mCi/mmol), [$^{14}$C]caffeine (49·3 mCi/mmol) and [$^{3}$H]testosterone (49·0 Ci/mmol) were obtained from New England Nuclear (Boston, MA, U.S.A.). The radiochemical purity of each compound was determined by the manufacturer to be at least 98%.

Gels were made of the liquid vehicles (ethylene glycol and water) using Carbopol 940 (B.F. Goodrich, Cleveland, OH, U.S.A.), so that compounds could remain on the skin in solution during the *in vivo* measurements. For consistency, gels were also used in the *in vitro* studies. The ethylene glycol gels were prepared by making initially a 1% solution of the Carbopol in ethylene glycol (with approximately 3 h of stirring for dissolution). The test substance was then added to the solution, and a gel was formed following the addition of 10 μl of triethanolamine (J.T.Baker Chemical Company, Phillipsburg, NJ, U.S.A.) per ml of ethylene glycol. The water gel was prepared with 1·5% Carbopol 940, and again a 3 h dissolution period was required. To form the gel, the solution was neutralized with 10% NaOH.

### In vitro *absorption*

*In vitro* studies with excised human abdominal skin were performed at the Food and Drug Administration, using a flow-through diffusion cell system (Bronaugh & Stewart, 1985). Cadaver skin was obtained within 24 h of death and was used immediately or stored in the frozen state for no more than 2 months. Each set of permeability values was obtained from samples of skin from at least two donors. Discs of skin were punched from sheets of skin prepared with a Padgett electro dermatome. The thickness of the membrane was approximately 350 μm, so that the membrane consisted of the epidermis and the upper papillary dermis.

The diffusion cells were assembled and placed in an aluminium holding block. Water was heated in a water bath at 35°C and pumped through the block to maintain the skin surface temperature at 32°C. Normal saline was pumped through the cell with a multichannel peristaltic pump (Manostat, New York, NY, U.S.A.) at a rate of 5 ml/h. The effluent was collected in a fraction collector until background levels of radioactivity were approached. The amount of radioactivity was determined with a Beckman LS 9000 scintillation counter (Beckman Instruments, Irvine, CA, U.S.A.).

Date were expressed in terms of a permeability constant ($K_p$), the lag time (from starting time until steady-state absorption was reached) and the percentage of the applied dose absorbed. When the steady-state absorption rate was obtained, the $K_p$ was determined by dividing the rate by the concentration of test material applied to the skin. Often only a pseudo-steady state was observed, since with finite dosing maximum rates are often transitory. The lag time was determined from the *x*-axis intercept of an extended straight line connecting the steady-state points in a cumulative plot of amount of compound absorbed *vs.* time.

### In vivo *absorption*

Adult male volunteers were recruited for the *in vivo* percutaneous absorption study performed at the University of Washington. A circular template of appropriate size was used to mark off two areas on each side of the abdomen just above the umbilicus. Two sites were used, rather than one, since it was somewhat easier to protect against rub-off, through the use of a non-occlusive guard, when sites of smaller dimension were used. The radioactive formulation was applied with a flat metal spatula to an area of 20–60 cm$^2$; the area of application was varied for each compound so that the radiation dosage to the skin at the test site was kept within the limits set down by the Nuclear Regulatory Commission.

All urinary output was collected throughout the course of the experiment until background levels were approached. These were collected as individual samples during the first 24 h and as pooled 8-h samples thereafter. In many of the experiments, the volunteers were requested to drink increased amounts of fluids during the first 8 h so that frequent urine samples could be obtained. Three-millilitre aliquots of urine were gelled with 4 ml of Aquasol-2 scintillation fluid and counted in a Beckman scintillation counter.

Each test compound was also administered i.v. to a group of volunteers so that urinary excretion after this route of administration could be determined. The fraction of the administered dose excreted in the urine was used to correct the values from the topical experiments for excretion by routes other than the urine. Total topical absorption was therefore determined by dividing the amount of compound excreted in the urine in the topical experiment by the fraction of the dose obtained in the urine in the i.v. experiment. The mean percentage (± SE) of the i.v. dose excreted in the urine was: benzoic acid, 96·2 ± 1·7; caffeine, 65·7 ± 5·1; and testosterone, 89·9 ± 3·7.

In both the *in vitro* and *in vivo* studies, excess material was removed from the application site at similar times. In the water gel experiments, excess compound was removed with a spatula and the skin surface was cleaned with soap and water at 24 h. In experiments with the other two vehicles, benzoic acid was removed at 24 h (since most absorption had occurred), and caffeine and testosterone at approximately 36 h. After excess material had been removed with a spatula, petroleum ether was used for cleansing when petrolatum had been applied; water was used to remove the remaining ethylene glycol gel.

### *Partition and solubility studies*

Human stratum corneum was prepared for the partition studies from full thickness skin. The epidermal layer was removed from the dermis after immersion of the skin in 60°C water for 60 s. This layer was then placed on filter paper soaked in trypsin solution (0·001%) and incubated overnight at 37°C. The partially-digested epidermal cells could then be removed with a cotton-tipped applicator, leaving a sheet of stratum corneum. The membrane was dried in a desiccator and the dry weight was used in subsequent experiments.

The stratum corneum/vehicle partition coefficient was determined by placing 5 mg of the membrane in a small screw-cap vial and adding 200 μl of vehicle containing the radiolabelled solute. Aliquots of the vehicle were analysed periodically to determine the loss of test compound. Blank samples (without stratum corneum) were run simultaneously to correct for any material adsorbed to the glass walls of the vials. Radioactivity not accounted for was assumed to have entered the stratum corneum. The volume of the membrane was determined by using the density of 1·32 (Scheuplein, 1966). The radioactivity per unit volume was calculated for the stratum corneum and vehicle at each sampling time.

Solubility determinations were made from saturated solutions prepared by stirring with excess material for 1 h. Petrolatum solutions were stirred on a hot plate and then centrifuged to remove undissolved material; radiolabelled compounds were used for convenience of analysis. For ethylene glycol and water solutions, excess material was removed by membrane filtration and compounds were quantitated by ultraviolet absorbance (Cary Model 118 spectrophotometer, Varian Associates, Palo Alto, CA, U.S.A.).

## RESULTS

*In vitro* permeability parameters are summarized in Table 1. The chemical dose and vehicle dose were not identical in all the studies because they were selected for purposes of making direct comparisons with the previously conducted *in vivo* measurements. Permeability comparisons are best made by examination of the permeability constants because the percentage

TABLE 1. Permeability parameters *(in vitro)* of benzoic acid, caffeine and testosterone in different vehicles

| Compound and vehicle | Compound dose ($\mu g/cm^2$) | Vehicle dose ($mg/cm^2$) | Permeability constant ($cm/h \times 10^4$) | % Dose/h at steady state | % Absorbed (total) |
|---|---|---|---|---|---|
| Benzoic acid | | | | | |
| Petrolatum (6) | 0·5 | 5 | — | — | 46·5 ± 5·9 |
| Petrolatum (11) | 2 | 25 | — | — | 41·8 ± 4·2 |
| Caffeine | | | | | |
| Petrolatum (12) | 2 | 25 | 7·2 ± 0·5 | 2·4 ± 0·2 (1·2)* | 61·8 ± 3·0 |
| Petrolatum (7) | 60 | 50 | 6·2 ± 0·3 | 1·4 ± 0·1 (1·1) | 40·6 ± 2·2 |
| Ethylene glycol gel (6) | 0·5 | 25 | 2·1 ± 0·5 | 0·8 ± 0·2 (3·2) | 32·2 ± 7·3 |
| Water gel (8) | 10 | 400 | 5·1 ± 0·6 | 0·1 ± 0·02 (4·1) | 4·7 ± 0·8 |
| Water gel (7) | 50 | 400 | 5·6 ± 0·7 | 0·1 ± 0·02 (5·6) | 5·1 ± 0·5 |
| Testosterone | | | | | |
| Petrolatum (20) | 2 | 25 | 2·8 ± 0·1 | 1·0 ± 0·1 (3·8) | 39·4 ± 1·2† |
| Ethylene glycol gel (5) | 2 | 25 | 1·8 ± 0·2 | 0·7 ± 0·1 (7·8) | 23·7 ± 2·0† |
| Water gel (8) | 1 | 400 | 67·1 ± 11·5 | 1·8 ± 0·2 (2·8) | 41·4 ± 6·8 |

Values are the mean ± SE. The number of determinations is given in parentheses.

* Lag time (h) in parentheses.

† Significant difference from *in vivo* values (Table 4), Student's *t*-test (two-tailed), $P < 0.05$.

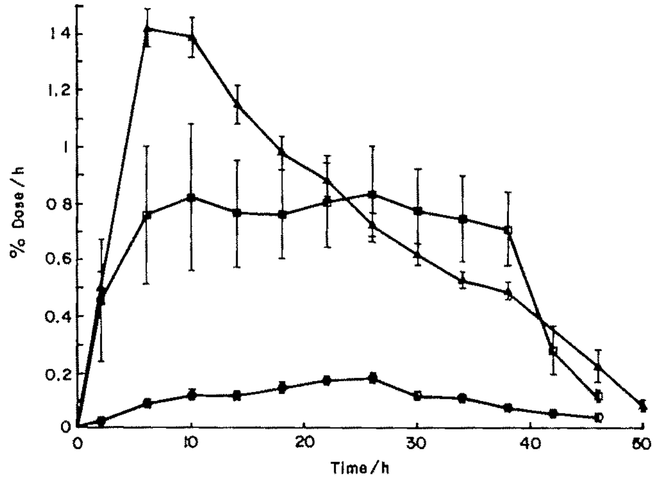

FIGURE 1. Effect of vehicles on caffeine absorption *in vitro*. Values are the mean (± SE) of 6–7 determinations. △ = petrolatum; □ = ethylene glycol gel; ○ = water gel

absorbed was influenced by the differing amounts of vehicle applied. Benzoic acid absorption was so rapid that a steady-state rate was not observed, and therefore a permeability constant could not be calculated. Because of the partial ionization of benzoic acid at the neutral pH of the water and ethylene glycol gels, absorption data obtained in these vehicles were difficult to interpret and are not reported.

Skin permeation ($K_p$ values) of caffeine was similar with the petrolatum and water vehicles. Only a transient steady-state absorption rate was observed from the petrolatum vehicle (Fig. 1). The relatively low percent absorption from the water gel was due to the large amount of gel applied (to reduce dead space in the application devices and thereby reduce evaporation of the vehicle). Caffeine was absorbed at a slower rate from the ethylene glycol gel, but the sustained absorption (Fig. 1) resulted in little difference in the total amount absorbed when compared to petrolatum (high dose).

Testosterone absorption was maximum from the water vehicle, with much lower $K_p$ values obtained when the compound was applied in petrolatum or ethylene glycol. The testosterone permeation rate reached a maximum at 10 h (water vehicle) and remained at that rate until the skin was washed at 24 h (Fig. 2). When petrolatum was used, the maximum rate occurred at approximately the same time but was more transient. Absorption increased at a slower rate with ethylene glycol as the vehicle.

The stratum corneum/vehicle partition coefficient ($K_m$) was determined for each compound (Table 2); measurements were made at more than one time to determine if equilibrium had been reached. Although equilibrium was approached after several days with ethylene glycol and water vehicles, $K_m$ values steadily increased up to 96 h with benzoic acid and caffeine in the petrolatum vehicle. Testosterone partitioned into stratum corneum most readily from an aqueous solution. The $K_m$ value in petrolatum increased when the concentration of testosterone was increased. Similar results were obtained at 24 h with caffeine in all three vehicles.

The vehicle solubility of the three test compounds (Table 3) gave additional insight into the chemical properties of the molecules. Caffeine and testosterone were reasonably soluble in petrolatum but their affinity for water and ethylene glycol differed greatly; caffeine was much

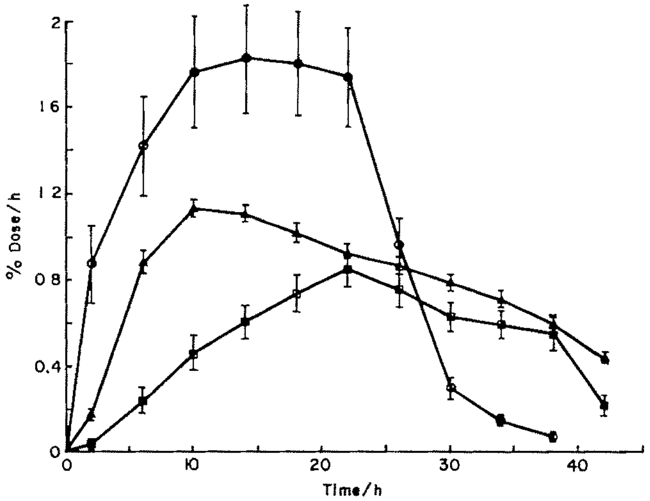

FIGURE 2. Effect of vehicles on testosterone absorption *in vitro* Values are the mean (± SE) of 5–20 determinations △ = petrolatum; □ = ethylene glycol gel; ○ = water gel.

TABLE 2. Stratum corneum/vehicle partition coefficients ($K_m$) for benzoic acid, caffeine and testosterone

| | Time (h) | | | | |
|---|---|---|---|---|---|
| Compound and vehicle | 6 | 24 | 48 | 72 | 96 |
| Benzoic acid | | | | | |
| Petrolatum | 5·3 ± 1·4 (4) | 20·1 ± 3·0 (8) | 38·9 ± 6·8 (7) | — | 63·1 ± 7·1 (8) |
| Caffeine | | | | | |
| Petrolatum | — | 1·5 ± 0·4 (7) | — | — | 3·3 ± 1·0 (7) |
| Ethylene glycol | — | 2·3 ± 0·2 (3) | 4·5 ± 1·3 (3) | 7·1 ± 1·0 (4) | — |
| Water | — | 3·5 ± 0·4 (4) | — | — | 3·2 ± 0·3 (4) |
| Testosterone* | | | | | |
| Petrolatum | — | 0 (14) | — | — | 1·6 ± 0·4 (14) |
| Ethylene glycol | — | 5·2 ± 0·5 (13) | — | — | 6·0 ± 1·0 (14) |
| Water | — | 47·4 ± 1·7 (14) | — | — | 57·9 ± 1·3 (14) |
| Testosterone† | | | | | |
| Petrolatum | — | 5·3 ± 0·7 (6) | — | — | — |
| Ethylene glycol | — | 3·3 ± 0·7 (6) | — | — | — |
| Water | — | 51·0 ± 4·0 (6) | — | — | — |

Values are the mean ± SE. The number of determinations is given in parentheses.

* [$^3$H]Testosterone concentration, 1 ng/25 μl.

† Unlabelled testosterone added to [$^3$H]testosterone to give concentration similar to permeability experiments (2 μg/25 μl).

TABLE 3. Vehicle solubility of benzoic acid, caffeine and testosterone

| | Solubility (g/l) | | |
|---|---|---|---|
| Compound | Petrolatum | Water | Ethylene glycol |
| Benzoic acid | 7·2 | — | — |
| Caffeine | 1·4 | 22·0 | 0·08 |
| Testosterone | 5·5 | 0·01 | 2·1 |

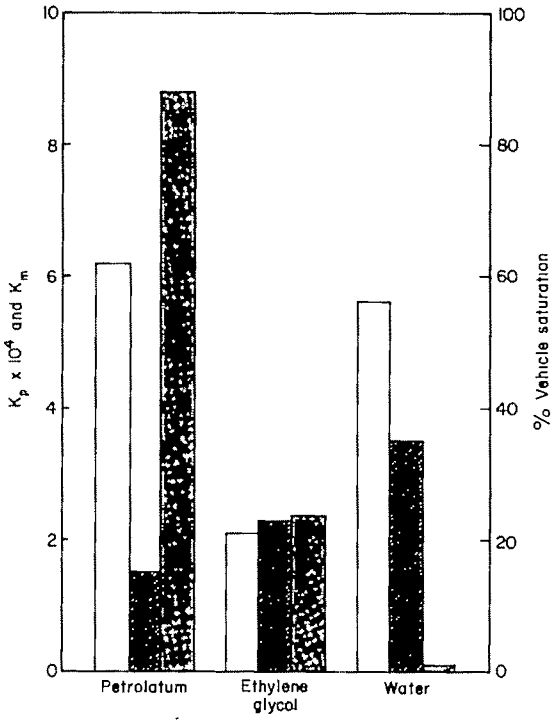

FIGURE 3. Comparisons of *in vitro* permeability parameters for caffeine. □ = $K_p \times 10^4$; ■ = $K_m$; ▨ = % vehicle saturation.

more soluble in water than in ethylene glycol and testosterone had preferential solubility in ethylene glycol.

The percent saturation of the vehicles in the permeability studies was calculated from the solubility data in Table 3. These values were compared with the respective $K_p$ and $K_m$ values for caffeine (Fig. 3) and testosterone (Fig. 4).

The absorption values obtained in the human *in vivo* experiments are reported in Table 4. The values for the percentage of the applied compound absorbed can be compared directly to the corresponding values calculated for the *in vitro* experiments (Table 1). The absorption

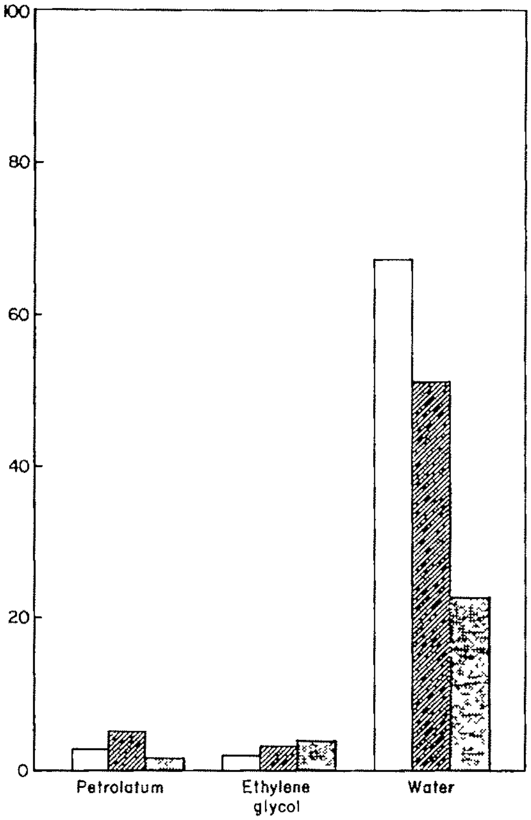

FIGURE 4. Comparisons of *in vitro* permeability parameters for testosterone. □ = $K_p \times 10^4$; ■ = $K_m$; ▨ = % vehicle saturation.

TABLE 4. Permeability parameters *(in vivo)* of benzoic acid, caffeine, and testosterone in different vehicles

| Compound and vehicle | Compound dose ($\mu$g/cm$^2$) | Vehicle dose (mg/cm$^2$) | % Dose/h (maximum) | % Absorbed (total) |
|---|---|---|---|---|
| Benzoic acid | | | | |
| Petrolatum (4) | 0·5 | 5 | 13·2 ± 0·7 (2·5)* | 60·6 ± 10·7 |
| Caffeine | | | | |
| Petrolatum (5) | 60 | 50 | 1·0 ± 0·2 (27) | 40·6 ± 6·1 |
| Ethylene glycol gel (4) | 0·5 | 25 | 1·7 ± 0·3 (26) | 55·6 ± 11·7 |
| Water gel (4) | 50 | 400 | 0·1 ± 0·03 (32) | 4·0 ± 0·5 |
| Testosterone | | | | |
| Petrolatum (3) | 2 | 25 | 1·7 ± 0·3 (11) | 49·5 ± 5·8 |
| Ethylene glycol gel (4) | 3 | 25 | 1·2 ± 0·4 (26) | 36·3 ± 0·4 |
| Water gel (4) | 1 | 400 | 2·1 ± 0·3 (10) | 49·2 ± 4·7 |

Values are the mean ± SE. The number of determinations is given in parentheses.

Absorption values were corrected for total absorption by using the appropriate i.v. correction factor.

* Time (h) of occurrence of maximum rate in parentheses.

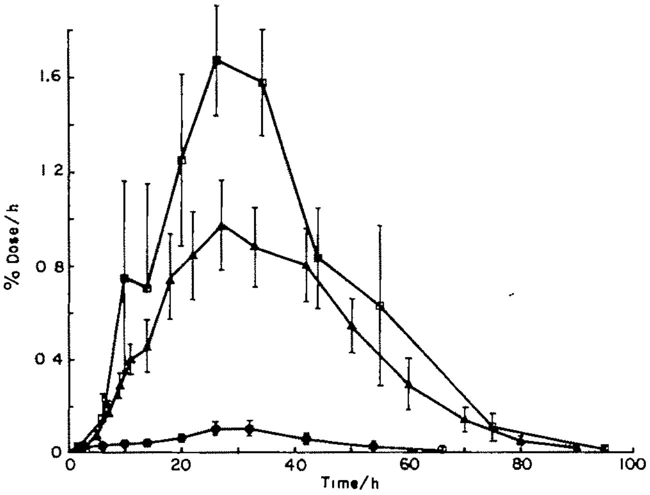

FIGURE 5. Effect of vehicles on caffeine absorption *in vivo*. Values are the mean (± SE) of 4–5 determinations. △ = petrolatum; □ = ethylene glycol gel; ○ = water gel.

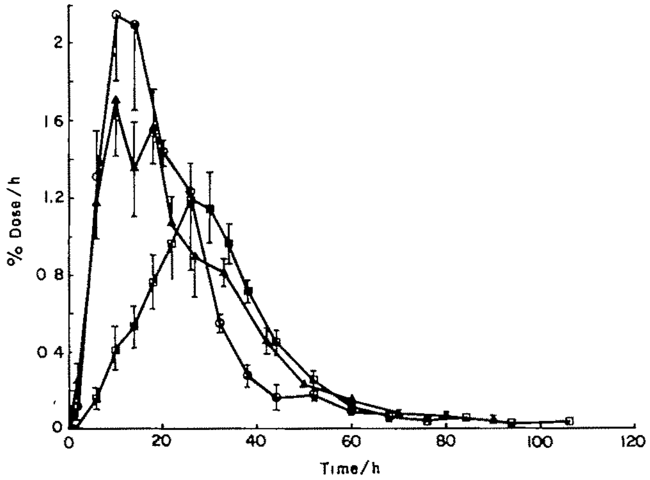

FIGURE 6. Effect of vehicles on testosterone absorption *in vivo*. Values are the mean (± SE) of 3–4 determinations. △ = petrolatum; □ = ethylene glycol gel; ○ = water gel.

profiles have been calculated for caffeine (Fig. 5) and testosterone (Fig. 6). Frequently, good agreement was obtained between the amount and time of maximum *in vivo* absorption and the corresponding *in vitro* data (Figs 1 and 2).

## DISCUSSION

### In vitro *experiments*

In the *in vitro* studies with caffeine (Table 1), permeation from petrolatum and water vehicles was carried out at different dosage levels and, with petrolatum, at different vehicle thicknesses.

The respective permeability constants were unaffected by dose, since $K_p$ values are independent of concentration at low dosage levels. Vehicle thickness was also without substantial effect on $K_p$ values because excess test compound was present at both doses of petrolatum. The total percentage of caffeine absorbed was unchanged when the compound dose was increased five-fold in the water gel. The effect of compound dosage changes on caffeine and benzoic acid absorption from petrolatum is difficult to interpret since the vehicle dosage was also changed.

The partitioning of compounds from vehicle to skin ($K_m$) was found to be a determining factor in rates of absorption (Figs 3 and 4). The $K_m$ values obtained at 24 h of equilibration were used in these comparisons because values at longer times would be less representative of the partitioning occurring during the skin absorption experiments. An exception was found with caffeine; here the $K_p$ was slightly higher from petrolatum than from the water vehicle, but the $K_m$ was two-fold lower. The percent saturation of the petrolatum vehicle with caffeine, however, was much higher than water, which could explain the higher petrolatum $K_p$ value. The greater percent saturation should also have resulted in a higher $K_m$ in petrolatum, but other factors such as vehicle effects on the structure of the stratum corneum may be involved. In Table 2, the $K_m$ values determined in petrolatum were still increasing even after the 4 days allowed to achieve equilibrium.

The $K_p$ value for caffeine was lowest in ethylene glycol and the caffeine solution was least saturated in this vehicle (Fig. 3). The $K_m$ value was intermediate between that in petrolatum and that in water, and it also increased with time. The effect of ethylene glycol on the integrity of skin may be questioned. Zatz (1983), however, concluded that the influence of a similar compound, propylene glycol, on benzocaine permeability of hairless mouse skin is not due to an interaction of the vehicle with the skin.

With testosterone (Fig. 4), good agreement between $K_p$, $K_m$, and percent saturation of vehicles was obtained. The penetration of testosterone was markedly increased by application in the water vehicle. The difference in absorption from the other vehicles was reflected clearly in the $K_m$ values. The low solubility of testosterone in water (Table 3) resulted in a highly favourable partitioning into the stratum corneum. Initial $K_m$ studies (Table 2) were conducted with tritium-labelled testosterone at a much lower concentration than that used in the permeability experiments. When these studies were repeated at the higher testosterone level, $K_m$ values in the same rank order as the $K_p$ values were obtained. The effect of concentration on the stratum corneum/vehicle partition coefficients for a series of hair dyes has recently been described by Bronaugh and Congdon (1984).

### In vivo *and* in vitro *comparisons*

The percentages of the compounds absorbed *in vitro* (Table 1) were generally in agreement with the corresponding values obtained *in vivo* (Table 4). Significantly lower values were obtained for testosterone absorption from both the petrolatum and ethylene glycol gel vehicles. No significant differences were found in caffeine absorption with any of the three vehicles, suggesting that differences in the comparisons were not due to vehicle effects. There was a trend towards lower values in the *in vitro* studies; in only one instance (caffeine in the water gel) was the absorption *in vitro* greater than that in the *in vivo* measurements. One can speculate on several possible reasons for this result. The *in vitro* studies were conducted at a skin temperature of 32°C, which was determined by the investigator to be the average forearm skin temperature of laboratory personnel. A higher skin temperature might be expected on the abdominal application site covered by the protective device and clothing. In addition, some error will inevitably be introduced by the use of the i.v. correction factor in the *in vivo* studies. In spite of this, there may be no better way to estimate human percutaneous absorption of compounds excreted predominantly in the urine. Shah and Guthrie (1983), using rats, obtained good agreement between absorption measurements by using the i.v. correction factor and a direct method involving sacrifice of the test animals.

The rates of absorption (% dose/h) measured during the *in vitro* studies (Figs 1 and 2) attained a pseudo-steady state and then declined after cleansing of the surface of the skin, if not before. The *in vivo* absorption profiles (Figs 5 and 6) from urinary excretion data (corrected with the i.v. factor) generally showed a more transient maximum absorption rate because of the pharmacokinetic processes involved. An *in vivo-in vitro* comparison shows that the rates were generally higher in the *in vivo* studies but by less than a factor of 2. When caffeine was applied in petrolatum, however, the maximum absorption rate was also transient in the *in vitro* study and was slightly higher than in the *in vivo* experiment. The delay before appearance of a maximum rate was longer in the *in vivo* studies, again presumably because of the pharmacokinetic considerations. This longer delay *in vivo* is reflected in a comparison of the lag times from *in vitro* studies (Table 1) with the time of occurrence of maximum *in vivo* rate (Table 4).

It may be concluded that reasonable agreement occurred between the *in vivo* and *in vitro* studies. The vehicle effects on percutaneous absorption can be explained in terms of the solubility properties of the penetrant and its stratum corneum/vehicle partition coefficient.

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# Monoclonal antibodies against epidermal cell surface and dermal-epidermal junction: antigens which may be epidermal cell spreading factors

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## SUMMARY

Two monoclonal antibodies which reacted with the epidermal cell surface (SF-1) and the dermal-epidermal-junction (SF-2), respectively, were obtained by immunizing mice with partially-purified human epibolin. The corresponding antigens were partially purified from fetal bovine serum by affinity chromatography using these antibodies. SDS-polyacrylamide gel electrophoresis showed that these antigens contained polypeptide components with molecular weights different from that of epibolin (mol. wt. 65 000 daltons); SF-1 antigen had a 68 000 dalton main component, and SF-2 antigen a broad 58 000–61 000 dalton main component. Both of these partially-purified antigens promoted the spreading of dissociated pig epidermal cells. SF-2 antigen also promoted the spreading of Pam cells (a murine keratinocyte line). The results suggest that proteins capable of promoting epidermal cell spreading may be present on the epidermal cell surface and at the dermal-epidermal junction. However, their physiological role in keratinization remains to be elucidated.

Cell spreading and attachment to the substratum is considered to be central to the survival of the majority of cells in *in vitro* culture. Recently, rapid progress has been made in the identification of molecules which mediate cell spreading and attachment, including fibronectin, laminin and chondronectin (Kleinman, Klebe & Martin, 1981; Hayman & Yamada, 1982; Barnes & Silnutzer, 1983). 'Serum spreading factor(s)' purified by several investigators (Grinnell, Hays & Minter, 1977; Whateley & Knox, 1980; Barnes & Silnutzer, 1983) are molecules present in serum that mediate cell spreading and that are distinct from the above molecules in their biochemical, immunological and biological properties. Epibolin, described by Stenn (1981), is a serum spreading factor which mediates epidermal cell spreading, although its exact properties

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have not been elucidated fully. In order to characterize epibolin in more detail, we prepared monoclonal antibodies using, as an antigen, an intermediate product from purification of this compound from human serum.

We describe here the properties of two monoclonal antibodies.

## METHODS

### *Preparation of monoclonal antibody*

A BALB/c mouse was immunized by injecting partially-purified epibolin (step 4 material) (EPS 4), a gift from Dr K.S.Stenn, Yale University, New Haven, CT, U.S.A. (Stenn, 1981). EPS 4 (250 μg) was emulsified in complete Freund's adjuvant and injected intraperitoneally, followed by a repeat injection without adjuvant one week later and a booster injection 2 weeks later. Three days after the booster injection, splenocytes from the immunized mouse were fused with SP2/0-Ag14 mouse myeloma cells according to the method of Köhler and Milstein (1975). Supernatants from cultures of the resulting colonies were screened for antibodies to epibolin by enzyme-linked immunosorbent assay (ELISA) (Kirkegaard & Perry Laboratories Inc., Gainthersburg, MD, U.S.A.), the antigen used to coat the wells being EPS4. If epibolin acts on epidermal cells, then the antibody against it may bind to these cells. Colony culture supernatants, therefore, were further screened by indirect immunofluorescence (IIF) staining of human and pig epidermis.

### *IIF tests on human and pig epidermis*

Cryostat sections of human and pig skin (4 μm) air-dried on gelatin-fixative (Harleco Inc., Gibbstown, NJ, U.S.A.) were incubated with culture supernatants for 30 min at room temperature, washed with phosphate buffered saline (PBS) and incubated with fluorescein isothiocyanate (FITC) conjugated goat antimouse IgG or IgM (Tago Inc., Burlingame, CA, U.S.A.) for 30 min, followed by washing with PBS. The sections were mounted in glycerol-PBS mixture and examined under a Leitz fluorescence microscope. As a control, culture supernatant from SP2/0-Ag14 myeloma cells was used as the first antibody.

### *IIF test on dissociated pig epidermal cells*

Two positive culture supernatants as evidenced by ELISA and IIF tests on human and pig epidermis were further examined by an IIF test on dissociated pig epidermal cells. Skin slices were obtained from domestic pigs weighing about 7–10 kg with a Castroviejo keratotome set at 0·2 mm. The epidermal cells were prepared by treatment of the skin slices with 0·05% trypsin mixed with 0·02% ethylene diaminetetraacetic acid (Gibco Laboratories, New York, NY, U.S.A.), followed by agitation of both separated epidermis and dermis (Liu & Karasek, 1978). $5 \times 10^5$ cells were incubated with the culture supernatants, concentrated five-fold with minicon B 15 concentrator (Amicon Corp., Danvers, MA, U.S.A.), in a 96 well microtitre plate for 30 min at 4°C. After washing three times with PBS, cells were incubated with FITC-conjugated goat antimouse IgG or IgM (Tago Inc., Burlingame, CA, U.S.A.) for 30 min. After washing five times with PBS, ethidium bromide 1 μg/ml in PBS was used to stain non-viable cells. As a control, culture supernatant from SP2/0-Ag14 myeloma cells five-fold concentrated was used as the first antibody.

### *Affinity chromatography*

Ascitic fluids were obtained from pristane-primed BALB/c mice injected intra-peritoneally with the hybrid cells. The antibody was precipitated with 45% ammonium sulphate. This precipitation step was repeated three times followed by dialysis against PBS. The antibody was then coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.). A short column, 1·0 × 6·5 cm, was prepared and fetal bovine serum diluted 1:2 with PBS was applied. After thorough washing with PBS, the antigen was eluted with glycine-HCl buffer (pH 2·5) immediately neutralized with Tris and dialysed against distilled water.

The antigen was further concentrated by lyophilization. Protein concentration was measured using Bio-Rad protein assay reagent (Bio-Rad Laboratories, Richmond, CA, U.S.A.).

### *Gel electrophoresis*

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) was carried out according to the method of O'Farrell (1975) using 10% acrylamide.

### *Cell spreading assay*

Pig skin slices were obtained and separated as described above, except that to obtain a basal cell rich fraction, the separated dermis only was agitated (Liu & Karasek, 1978) in RPMI 1640 medium with 0·5% soybean trypsin inhibitor (Sigma, St. Louis. MO, U.S.A.).The cells were washed twice with serum-free RPMI 1640 medium to yield a basal cell rich fraction (above 90% basal cells) with 95% viability. Basal cells were counted according to the criterion of Vaughan and Bernstein (1971). Approximately 1·5 × 10$^4$ cells suspended in serum-free RPMI 1640 medium were placed in each well of 96 well tissue culture plates (Costar, Cambridge, MA, U.S.A.) and pre-incubated for 2 h in a humidified incubator with 5% CO$_2$. The medium was then discarded to remove non-adherent cells, and replaced by the test medium containing various amounts of the partially-purified antigens (Stenn, 1981). After 4 h incubation in the test medium, the cells were fixed with glutaraldehyde (2·5%) and the number of spread cells per 100 non-keratinized cells was counted under a phase contrast microscope. Cells which lost their round shape were defined as spread. Spreading assay of Pam cells (a spontaneously transformed murine keratinocyte cell line) using 1·8 × 10$^4$ cells/well, was performed in a similar way after harvesting dissociated Pam cells in serum-free medium from cells maintained in PMI 1640 medium with 10% fetal bovine serum.

## RESULTS

### *Production of monoclonal antibodies*

In the hybridization experiment, 221 out of 960 microtitre wells produced colonies. Screening of the culture supernatants from these colonies yielded two antibodies (SF-1 and SF-2) which reacted positively with human and pig epidermis, as evidenced by both ELISA and IIF tests. The hybrid cell lines which secreted these antibodies were cloned further by the limiting dilution technique. The isotypes of the antibodies were determined by the Ouchterlony technique; SF-1 was class IgM and SF-2 was class IgG$_1$.

### *IIF tests*

On cryostat sections of both human and pig skin, SF-1 showed dense punctate fluorescence on epidermal cell membranes and in the intercellular spaces of the entire epidermis and the hair follicles (Fig. 1). SF-2 showed similar but weaker punctate fluorescence only at the epidermal-dermal junction of human and pig skin (Fig. 2). Control culture supernatant gave no staining at

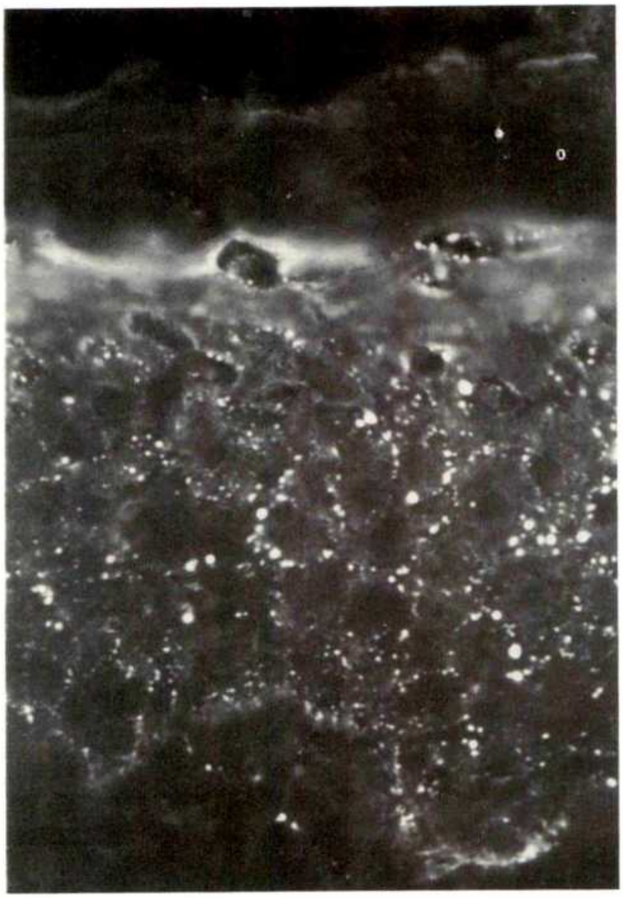

FIGURE 1. Punctate staining of human epidermal cell membranes and the intercellular spaces demonstrated with SF-1 by IIF (original × 200).

these sites. With dissociated pig epidermal cells, SF-1 stained the membrane of about half of the viable cells in a punctate or linear fashion (Fig. 3). Control culture supernatant and SF-2 gave no staining.

### *ELISA*

Specificity of SF-1 and SF-2 after limiting dilution against EPS 4 was confirmed by ELISA (Table 1). It was also shown that these antibodies did not react with bovine serum albumin.

### *SDS-PAGE*

The partially-purified SF-1 antigen and SF-2 antigen were analysed by SDS-PAGE. The EPS 4 was found to have several protein components with molecular weights about 54 000 daltons (Fig. 4). SF-1 antigen had a major polypeptide band of 68 000 daltons and two minor bands of 59 500 and 56 000 daltons. SF-2 antigen had a major band of polypeptides with molecular weights ranging from 58 000–61 000 daltons and a minor band of 68 000 daltons.

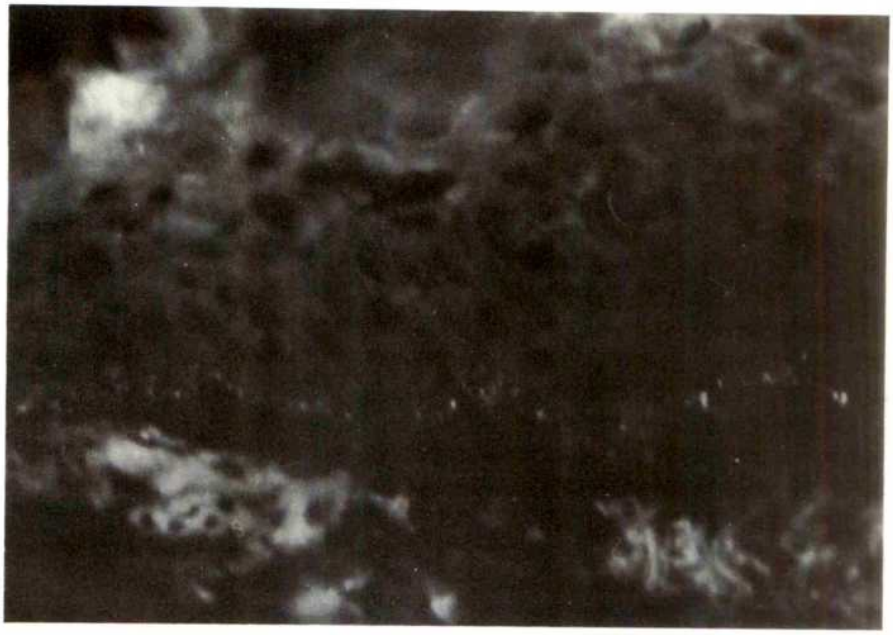

FIGURE 2. Punctate staining of human dermal-epidermal junction demonstrated with SF-2 by IIF technique (original × 100).

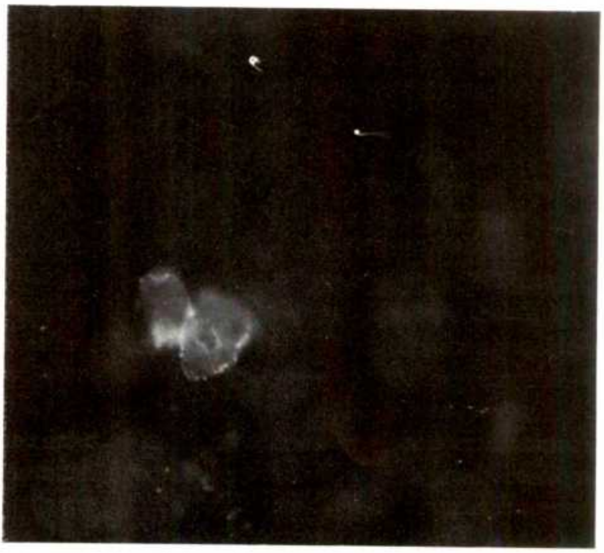

FIGURE 3. Labelling of trypsin-dissociated pig epidermal cells with SF-1 (culture supernatant concentrated five-fold) visualized by FITC conjugated antimouse IgM. Cells with punctate fluorescence on the surface can be seen (original × 200).

TABLE 1. Results of ELISA

| Antigen | Antibody | Absorbance at 410 nm |
|---|---|---|
| EPS 4 | SF-1 | 0·20 |
| EPS 4 | SF-2 | 0·27 |
| EPS 4 | SP2/0-Ag14 | 0·08 |
| BSA | SF-1 | 0·08 |
| BSA | SF-2 | 0·09 |
| BSA | SP2/0-Ag14 | 0·08 |

5 μl quantities of antigens were used. Culture supernatants after limiting dilution were used as antibodies

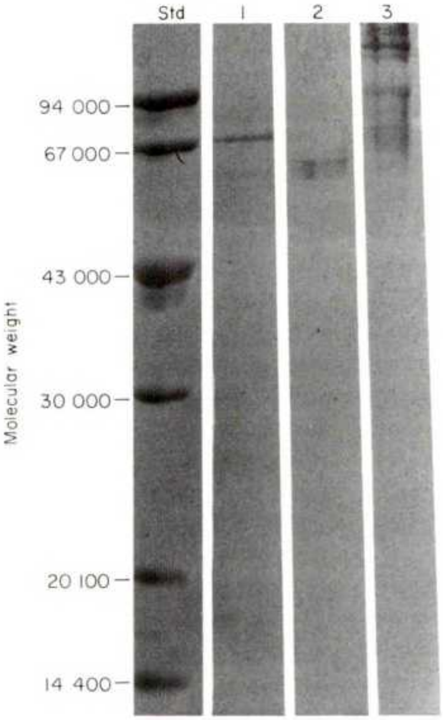

FIGURE 4. SDS-PAGE of SF-1 antigen and SF-2 antigen partially purified by affinity chromatography stained with 0·25% Coomassie brilliant blue R-250. Lane 1: SF-1 antigen; Lane 2: SF-2 antigen; Lane 3: EPS 4. Left-hand lane (Std): molecular weight standards: phosphorylase b (94 000), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), soybean trypsin inhibitor (20 100), α-lactalbumin (14 400).

### *Cell spreading assay*

As shown in Figure 5, partially-purified SF-1 and SF-2 antigens both promoted the spreading of dissociated pig epidermal cells in a dose-dependent manner even at concentrations below 10 μg/ml. The partially-purified SF-2 antigen promoted the spreading of dissociated Pam cells, while the partially-purified SF-1 antigen did not (Fig. 6).

## DISCUSSION

Two interesting monoclonal antibodies were obtained in this study. SF-1 stained the intercellular spaces of the epidermis in cryostat sections of human and pig skin in IIF tests. The fact that SF-1 also stained dissociated pig epidermal cell surfaces indicates that the corresponding antigen exists on epidermal cell membranes rather than in the intercellular spaces of the epidermis. SF-2 stained the dermal-epidermal junction.

Both antigens promoted the spreading of pig epidermal cells, and the antigen corresponding to SF-2 also promoted the spreading of Pam cells. However, it remains to be determined whether these partially-purified antigens contain epibolin. As shown by SDS-PAGE, the

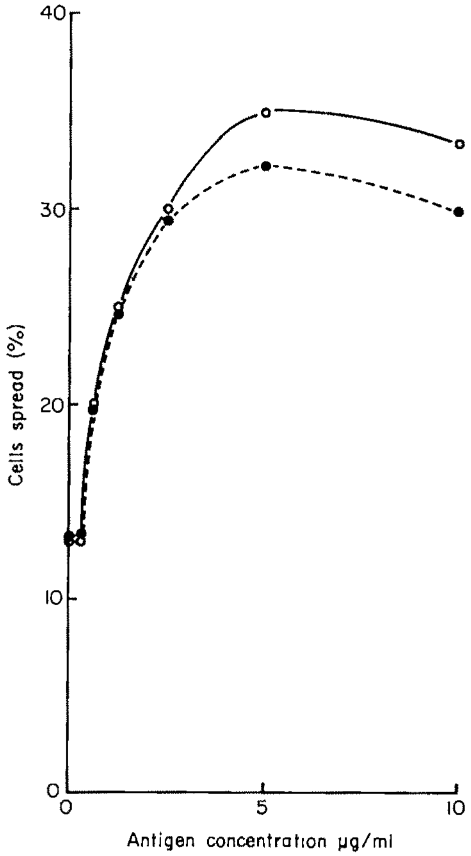

FIGURE 5. Spreading of dissociated pig epidermal cells induced by partially purified SF-1 (●) and SF-2 (○) antigens. Points are the means of two experiments, in which more than 300 cells were counted.

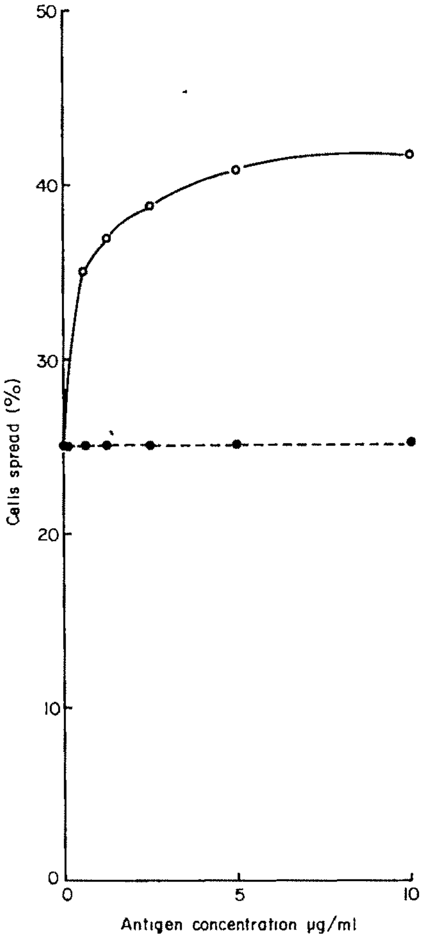

FIGURE 6. Spreading of dissociated Pam cells induced by partially-purified SF-1 (●) and SF-2 (○) antigens. Points are the means of two experiments, in which more than 300 cells were counted.

molecular weights of the polypeptide components of SF-1 antigen are 68 000 daltons (main band), 59 000 and 56 000 daltons (minor bands), and those in SF-2 antigen are 58 000–61 000 daltons (main bands) and 68 000 daltons (minor bands), while that of epibolin is 65 000 daltons (Stenn, 1981). Thus, it seems unlikely that these partially-purified antigens contained epibolin. There are several serum spreading factors with different molecular weights other than epibolin. Serum spreading factor purified from human serum by Barnes and Silnutzer (1983) has components with molecular weights of approximately 65 000 daltons (65 000–70 000) and 75 000 daltons (75 000–78 000) and that purified from fetal bovine serum by Whateley and Knox (1980), a molecular weight of approximately 62 000 daltons.

One possible explanation for the occurrence of several spreading factors with different molecular weights is that they have some common domain capable of promoting cell spreading. Another possibility is that there may be several spreading factors acting specifically upon different cell types. The fact that both partially-purified SF-1 and SF-2 antigens promote the spreading of epidermal cells suggests the existence of epidermal cell spreading factors on epidermal cell membranes and at the dermal-epidermal junction. Our results also suggest that the EPS 4 may also contain spreading factors other than epibolin. There are several large molecules capable of stimulating cell spreading or attachment or both, including fibronectin, laminin, chondronectin and type 4 collagen. On the basis of molecular weight, the epidermal cell spreading factors in SF-1 antigen and SF-2 antigen are distinct from other known large molecules (Foidart *et al.*, 1980; Sage, 1982).

Concerning the distribution of serum spreading factors in the body, Barnes *et al.* (1983) developed monoclonal antibodies to their spreading factor, and found it to be associated with human platelets. Hayman *et al.* (1983) also prepared monoclonal antibodies against a spreading factor, which they had partially purified from human serum by chromatography on glass bead columns (molecular weights 65 000 and 75 000 daltons) and found that it was present on loose connective tissue and on cultured fibroblasts. So far, epibolin has not been found to be associated with any particular tissue or organ.

The staining pattern of SF-1 is somewhat similar to that of pemphigus antibody. The molecular weight of pemphigus antigen, 130 000 daltons when reduced (Stanley *et al.*, 1982), is different from SF-1 and SF-2 antigens. However, identity between these antibodies remains to be determined. Aiba *et al.* (1983) developed a monoclonal antibody (AHS-4) reactive with epidermal cell membrane by using human epidermis as an antigen. The antigen, however, was not biochemically characterized.

SF-2 is similar to pemphigoid antibody in staining the dermal-epidermal junction. However, the staining pattern of SF-2 is different from that of pemphigoid antibody, the former being punctate and the latter linear. Moreover, the pemphigoid antigen is a large molecule with a molecular weight of approximately 220 000–240 000 daltons (Stanley, Woodley & Katz, 1984), like other constituents of the dermal-epidermal junction such as fibronectin, laminin, type 4 collagen and heparan sulphate proteoglycans. Our results suggest that SF-2 antigen is different from pemphigoid antigen and other known constituents of the dermal-epidermal junction. Two recently developed monoclonal antibodies, KF-1 (Breathnach *et al.*, 1983) and AHS-7 (Aiba *et al.*, 1983) stain the dermal-epidermal junction but their antigens are not characterized biochemically. The linear staining pattern of these antibodies suggests that they are different from SF-2. The cell spreading factor may be a previously undescribed constituent of the dermal-epidermal junction.

In our culture system, even the control cells in serum-free medium spread to some degree. A similar phenomenon was observed by Barnes *et al.* (1983) and they reported that spreading of human fibroblasts in the absence of exogenous promoting factors might result from the promoting action of fibronectin synthesized by the cells. Recently, it was shown that epidermal cells synthesize fibronectin (Kubo *et al.*, 1984; O'Keefe *et al.*, 1984). Thus, fibronectin synthesized by epidermal cells might explain our observation, although the molecular weight of fibronectin is 220 000.

The staining of epidermal cell surfaces by SF-1 and by SF-2 suggests the biosynthesis of spreading factors by epidermis itself. The physiological as well as the pathological significance of these spreading factors remains to be investigated.

## ACKNOWLEDGMENT

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# Lichen planus in relation to occupational and non-occupational exposure to chemicals

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## SUMMARY

Lichen planus-like eruptions are known to be caused by occupational exposure to colour film developing agents that are derivatives of *p*-phenylenediamine (PPDA), e.g. CD-2 and CD-3. To elucidate whether such chemicals are a cause of lichen planus in patients at a department of general dermatology, 119 lichen planus patients were interviewed about occupational and non-occupational exposure to chemicals. Patch testing with CD-2, CD-3, PPDA and IPPD was performed in 49. No occupational risk group for lichen planus could be identified and no case of contact allergy to the tested substances was found. Patch testing with these substances is only recommended when there is a history of exposure.

Lichen planus is a relatively common disease that affects skin and mucous membranes. There are many theories about the causes, including infective, auto-immune, psychogenic and genetic. Eruptions similar to lichen planus may be produced by certain drugs, by graft-versus-host reactions and by colour developing agents (Samman, 1979).

The colour film developing agents, e.g. CD-2 and CD-3, are derivatives of *p*-phenylenediamine (PPDA) and since 1958 (Buckley, 1958) it has been known that they can cause contact allergy, eczema and lichenoid reactions or lichen planus (for further references *see* Lidén, 1984). In most clinical cases of lichenoid reaction caused by colour developing agents, patients give positive patch test reactions. In many cases, these show lichenoid characteristics on histological examination (unpublished observations). There are also reports of lichenoid reactions, with positive patch test reactions, from black stockings and PPDA (Meneghini, 1971) and *p*-isopropylamino-diphenylamine (IPPD) in rubber (Ancona, Monroy & Fernández-Diez 1982; Calnan, 1971). Different aetiological factors for oral lichen planus have been discussed, and in one report there is an association between a high incidence of contact allergy to mercury and oral lichen planus (Finne, Göransson & Winckler, 1982). In addition, the results from some immuno-histological studies have suggested that a cell-mediated immune reaction may be involved in the pathogenesis of lichen planus (Tjernlund-Malmnäs, 1980; Bjerke & Matre, 1983).

The aim of the present study was to determine if exposure to colour developing agents (e.g.

CD-2 and CD-3) was a cause of lichen planus in patients at a department of general dermatology and if chemical exposure, primarily to PPDA and its derivatives CD-2, CD-3 and IPPD, was associated with the onset or aggravation of lichen planus. Contact allergy to these chemicals among lichen planus patients was also studied and the possibility of provoking lichen planus-like eruptions by patch testing lichen planus patients with these substances was investigated.

## METHODS

### *Patients*

The investigation included 130 fit subjects (51 female, 79 male) diagnosed as having lichen planus. All case records of women and men from 1982 at the Departments of Dermatology, Karolinska Hospital and Danderyd Hospital, and of men from 1983 and 1984 at Karolinska Hospital with this diagnosis, were reviewed. Patients who had records of lichenoid eruptions on the wrists, forearms, ankles or lower legs were included. Patients older than 68 and younger than 17 years of age, with an uncertain diagnosis, with mainly oral or genital lichen planus, or distribution of lesions without involvement of wrists, forearms, ankles or lower legs, were excluded.

All subjects who fulfilled the criteria for inclusion in the study received a letter explaining the background and design of the study and a request for their participation. All who agreed to participate were contacted.

### *Questionnaire*

A questionnaire was sent to the participants. The questions related to occupation, time and site of onset of lichen planus, and course. They were asked about contact with film developers, dark textiles, hair dyes, and rubber objects during the month prior to the onset of lichen planus or during the illness. They were also asked if any of these or other contacts were suspected of having caused or aggravated the skin disease.

### *Patch testing*

Patch testing was done with *p*-phenylenediamine (PPDA), *p*-isopropylamino-diphenylamine (IPPD) and the colour developing agents CD-2 and CD-3 (Fig. 1). The test substances were the following: PPDA 1% in petrolatum on subjects recorded in 1982 and 0·5% on subjects recorded in 1983 and 1984; IPPD was tested at 0·1% in petrolatum; CD-2 and CD-3 were tested at 1% in petrolatum. PPDA and IPPD were supplied by Trolab (Hellerup, Denmark) and later by Hermal-Chemie (Reinbek-Hamburg, FRG). CD-2 and CD-3 from Kodak were of commercial quality with unknown purity. They were prepared at the Karolinska Pharmacy, Stockholm, Sweden. Al-test® (Imeco, Södertälje, Sweden) and Scanpor® surgical tape (Norgeplaster, Kristiansand, Norway) were used. The test sites on the back were marked with a special pen (Crystal Violet 1%, alcohol (95%) 50%, silver nitrate 20%, purified water 30%). The patches were removed after 48 h and readings were made 72 h after application and 14–16 days later. The patients themselves had during this time filled in the marks with a marking pen when necessary. Those patients recorded in 1982 who had had any test reaction and were available were re-tested one year later with the particular substance.

### *Interview and clinical examination*

At the personal interview and clinical examination, the answers to the questionnaire were

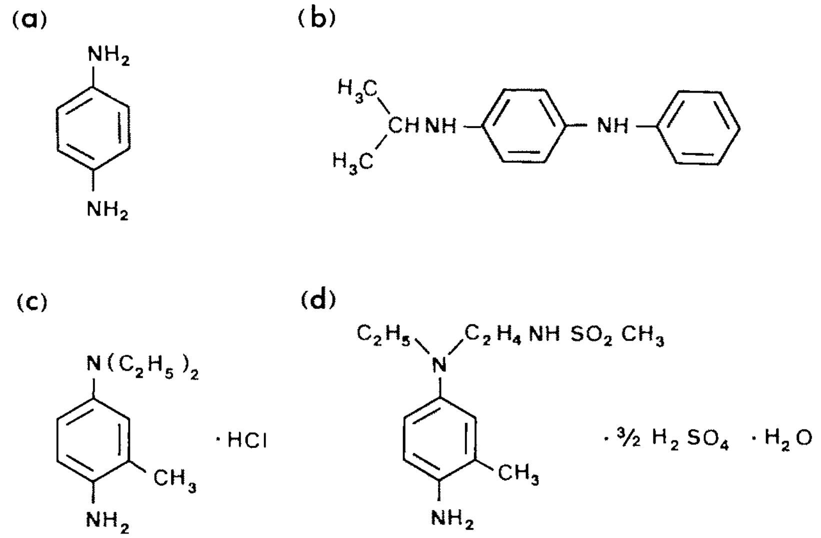

FIGURE 1. (a) PPDA (*p*-phenylenediamine); (b) IPPD (*p*-isopropylamino-diphenylamine), (c) CD-2 (4-N,N-diethyl-2-methyl-1,4-phenylenediamine monohydrochloride); (d) CD-3 (4-(N-ethyl-N-2-methan-sulphonylaminoethyl)-2-methyl-1,4-phenylenediamine sesquisulphate-monohydrate).

checked. The remembered maximum distribution and the present localization of active lesions and residual pigmentation from lichen planus were marked on a diagram. Questions were asked about nail involvement, oral and genital lesions, and the Koebner phenomenon. The nails and oral cavity were examined in all subjects.

### *Telephone interview*

Those who had not answered the invitation or could not participate in the patch testing were asked to participate in a telephone interview. The same questionnaire was sent to the subjects and the answers were checked in a telephone interview. At most, two reminders were sent.

### *Occupational classification*

Classification of occupations was done according to the Swedish application of the International Standard Classification of Occupations (Nordisk yrkesklassificering, 1978) into occupational groups (2-digit level). The occupation at the time of the study was recorded. In only a few cases did it differ from the occupation at onset of the disease. The result of this classification was compared with the occupational classification (2-digit level) in the 1980 Population and Housing Census for the County of Stockholm (Population and Housing Census 1980, personal communication, 1985).

## RESULTS

Patient details are shown in Table 1. Of the 130 patients who fulfilled the criteria for inclusion in the study, 79 participated in the patch testing and 40 in a telephone interview. Punch biopsies for histopathology had been taken from 69 of the participants and all showed lichen planus. Table 2 shows the number of years that had elapsed between the onset of lichen planus and the study. Disease activity on the trunk and extremities at the examination is shown. Active lesions were found in 47 of the 79 subjects examined. Table 3 shows the distribution of lichen planus on

TABLE 1. Patients included in the study

| | Total | Women | Men |
|---|---|---|---|
| Recorded | 311 | 129 | 182 |
| Eliminated | 181 | 78 | 103 |
| Wrongly recorded | 22 | 7 | 15 |
| Older than 68 or younger than 17 years | 45 | 23 | 12 |
| Uncertain diagnosis | 51 | 14 | 37 |
| Distribution criteria not fulfilled | 24 | 14 | 10 |
| Mainly oral or genital | 40 | 18 | 22 |
| Severely ill | 8 | 2 | 6 |
| Language difficulties | 1 | 0 | 1 |
| Selected for the study | 130 | 51 | 79 |
| Participated in patch testing | 79 | 40 | 39 |
| Participated in telephone interview | 40 | 11 | 29 |
| Dropped out | 11 | 0 | 11 |

Women recorded in 1982 and men in 1982–84.

TABLE 2. Duration of lichen planus prior to the study. Status of lesions on trunk and extremities in 79 subjects examined.

| | No. of subjects | | | |
|---|---|---|---|---|
| Duration (years) | Active lesions | Residual pigmentation only | Healed | Total |
| <1 | 11 | 5 | 0 | 16 |
| 1–2 | 13 | 11 | 3 | 27 |
| 2–5 | 11 | 6 | 2 | 19 |
| >5 | 12 | 3 | 2 | 17 |
| All | 47 | 25 | 7 | 79 |

the trunk and extremities. Residual pigmentation on the feet and lower legs was more common in men (16 cases) than in women (nine cases). The women reported lesions on the hands and forearms more often than the men (36 women and 26 men) and on the trunk (24 women and 17 men). The Koebner phenomenon had been noticed by 21 subjects, and in nine of them it persisted. Nail changes that could be connected with lichen planus were noticed in 45 subjects (increase in the longitudinal striations, but also ridging, thinning, pterygium formation, and shedding of nails). In 14 cases the changes were minor and non-diagnostic. The men especially had not noticed the changes themselves. Fifty-two had had oral symptoms or showed white streaks on the buccal mucosa or the inner surface of the cheeks. Fourteen subjects reported that they had had genital lichen planus.

TABLE 3. Localization of lichen planus on trunk and extremities in 79 subjects examined

| | No. of subjects | | |
|---|---|---|---|
| | Active lesions | Residual pigmentation | Stated maximum distribution |
| Hands, forearms | 16 | 32 | 62 |
| Upper arms | 4 | 3 | 22 |
| Feet, lower legs | 40 | 25 | 72 |
| Thighs | 3 | 3 | 7 |
| Trunk | 6 | 16 | 41 |

The 119 subjects represented 31 occupational groups (2-digit level) (Table 4). The sizes of these groups corresponded well with the statistics in the 1980 Population and Housing Census for the County of Stockholm. No occupational group was over-represented among the lichen planus patients. Table 5 gives details of contact during the month before the onset of lichen planus or during the illness, with film developers, dark textiles, hair dyes or rubber objects. The number of subjects who suspected that these contacts had aggravated the skin disease is also shown.

Seven of the 10 subjects who reported contact with film developers had occupational exposure—three X-ray assistants, one dental nurse, one photographer, one film director, and one graphic arts designer. The rest were amateur photographers. One photographer had spilled a can of film developer with Metol and hydroquinone (Fig. 2) on his arms and trunk one month before the onset of the lichen planus. The lesions healed in 2 months, but afterwards he had a rash on his hands from Metol contact. Patch tests with Metol and hydroquinone were negative.

The five subjects who experienced aggravation by dark textiles incriminated mourning clothes, black socks, dark blue pants and trousers. The three subjects who experienced problems with rubber mentioned rubber boots and elastic in underclothes. Ten subjects suspected aggravation by other miscellaneous contacts. A motor mechanic was accidentally drenched by a strong cleaning agent for tubines (Vecom B-80®) and a few weeks later lichen planus appeared on the sites of contact. A colour mixer's legs were splashed with alcohol used in the manufacture of dyes and some days later lichen planus appeared. A cleaner suspected aggravation by a strong acid cleaner (Hygilen®). A farmer suspected aggravation by a salt of formic acid (Kofa®). The others associated aggravation with welding, contact with warm gearbox oil, warm trousers and boots and some foodstuffs and liquors.

In Table 6 the test reactions at 72 h and 2 weeks after application of PPDA, IPPD, CD-2 and CD-3, are shown, including weak reactions not accepted as positive in the diagnosis of contact allergy. No positive reaction was found at the 72 h reading. Five sites showed slight irritation such as redness or small papules. At the second reading 2 weeks after application one positive reaction to PPDA was found, and four showed faint papules, and four redness. One year later, when six subjects were re-tested, Case 2 showed a positive reaction with redness, infiltration and papules to PPDA at 72 h. The weak reactions in Case 4 were reproduced, but not in the remaining cases.

Of the 11 cases that showed any sort of test reaction, seven had active lichen planus lesions,

TABLE 4. Occupations of the subjects in the lichen planus study and of the economically active population in the County of Stockholm taken from the Population and Housing Census 1980. Values are numbers of people and % of all occupations

| | | Lichen planus study | | Population and Housing Census 1980 | |
|---|---|---|---|---|---|
| Occupational classification | | no. | % | no. | % |
| 0 | Professional, technical and related work | 30 | 25·21 | 231 096 | 29·10 |
| 00 | Engineering | 6 | 5·04 | 57 035 | 7·18 |
| 04 | Health and nursing | 11 | 9·24 | 50 030 | 6·30 |
| 05 | Education | 7 | 5·88 | 45 489 | 5·73 |
| 08 | Literary and artistic | 2 | 1·68 | 16 906 | 2·13 |
| 09 | Other professional, technical and related work | 4 | 3·36 | 41 438 | 5·22 |
| 1 | Administrative and managerial | 1 | 0·84 | 33 444 | 4·21 |
| 10 | Government legislative and administrative | 1 | 0·84 | 13 757 | 1·73 |
| 2 | Bookkeeping and clerical work | 8 | 6·72 | 136 401 | 17·18 |
| 20 | Bookkeeping and cashier's work | 2 | 1·68 | 26 847 | 3·38 |
| 29 | Clerical and related work | 4 | 3·36 | 109 554 | 13·80 |
| 3 | Sales | 10 | 8·40 | 75 419 | 9·50 |
| 31 | Sales of insurance, real estate securities, business services | 1 | 0·84 | 6926 | 0·87 |
| 33 | Other sales | 9 | 7·56 | 62 888 | 7·92 |
| 4 | Agricultural, forestry and fishing | 4 | 3·36 | 8916 | 1·12 |
| 40 | Agricultural, horticultural and forestry management | 2 | 1·68 | 3776 | 0·48 |
| 41 | Agricultural, horticultural and livestock | 1 | 0·84 | 4208 | 0·53 |
| 44 | Forestry | 1 | 0·84 | 720 | 0·09 |
| 5 | Mining and quarrying | 1 | 0·84 | 614 | 0·08 |
| 50 | Mining and quarrying | 1 | 0·84 | 614 | 0·08 |
| 6 | Transport and communications | 11 | 9·24 | 55 902 | 7·04 |
| 60 | Ships' officers | 1 | 0·84 | 1034 | 0·13 |
| 63 | Rail and road transport | 6 | 5·04 | 27 005 | 3·40 |
| 65 | Post and telecommunications | 3 | 2·52 | 10 991 | 1·38 |
| 67 | Other transport and communications | 1 | 0·84 | 562 | 0·07 |
| 7–8 | Production | 28 | 23·53 | 132 221 | 16·65 |
| 71 | Sewing | 2 | 1·68 | 2387 | 0·30 |
| 75 | Engineering and building metalwork | 7 | 5·88 | 33 379 | 4·20 |
| 76 | Electrical and electronics | 1 | 0·84 | 18 621 | 2·35 |
| 77 | Wood work | 7 | 5·88 | 11 179 | 1·41 |
| 78 | Painting | 2 | 1·68 | 5379 | 0·68 |
| 79 | Other building and construction | 5 | 4·20 | 9559 | 1·20 |
| 82 | Food processing | 2 | 1·68 | 5209 | 0·66 |
| 87 | Operations monitoring, material handling | 2 | 1·68 | 5678 | 0·72 |
| 9 | Service | 21 | 17·65 | 109 412 | 13·78 |
| 90 | Civilian protective service | 3 | 2·52 | 11 239 | 1·42 |
| 91 | Housekeeping and related service | 9 | 7·56 | 52 771 | 6·65 |
| 93 | Caretaking and cleaning | 7 | 5·88 | 28 376 | 3·57 |
| 94 | Other service | 2 | 1·68 | 9820 | 1·24 |
| 99 | Workers reporting occupations unidentifiable or inadequately described | 5 | 4·20 | 6751 | 0·85 |
| All occupations | | 119 | 100·00 | 794 141 | 100·00 |

TABLE 5. Stated contact with film developers, dark textiles, hair dyes and rubber objects during the month before the onset of lichen planus or during the illness, among 119 subjects interviewed, and stated suspicion of detrimental effects

| | No. of subjects stating: | |
|---|---|---|
| | contact with | detrimental effects |
| Film developers | 10 | 1 |
| Dark textiles | 72 | 5 |
| Hair dyes | 13 | 0 |
| Rubber objects | 37 | 3 |

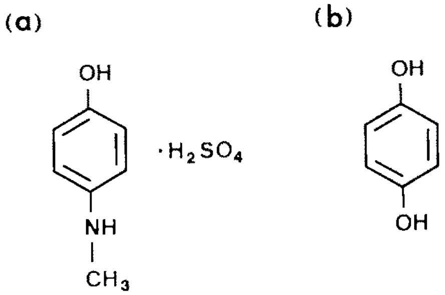

FIGURE 2. (a) Metol (*p*-methylaminophenol sulphate); (b) hydroquinone (1,4-benzenediol).

TABLE 6. Results of patch testing with PPDA, IPPD, CD-2 and CD-3 on 77* lichen planus patients

| | | Test reactions | | |
|---|---|---|---|---|
| | Case no. | 72 h | 2 weeks | No. of tests negative at 72 h and 2 weeks |
| PPDA | 1 | negative | faint papules | 74 |
| | 2 | negative | redness, infiltration | |
| | 3 | negative | faint papules | |
| IPPD | 4 | negative | redness, scaling | 72 |
| | 5 | redness | negative | |
| | 6 | redness | redness | |
| | 7 | small pustules | negative | |
| | 8 | few papules | negative | |
| CD-2 | 4 | negative | redness | 73 |
| | 7 | negative | redness | |
| | 9 | negative | few papules | |
| | 10 | few papules | negative | |
| CD-3 | 1 | negative | faint papules | 75 |
| | 11 | negative | faint papules | |

* Two subjects not participating in the reading at 2 weeks are not shown.

two residual pigmentation and two were healed. Only one (Case 6) suspected that the lesions were aggravated by external contacts, *viz.* dark blue pants.

In no case was current lichen planus aggravated by patch testing, nor did any typical lichen planus lesions develop at the test sites.

## DISCUSSION

There are more than 20 reports on more than 80 cases which show convincingly that occupational exposure to colour developing agents can cause lichenoid reactions, identical with or similar to lichen planus (for a review, *see* Lidén, 1984). This study was initiated to elucidate whether lichen planus in patients at a department of general dermatology may also be related to such exposure.

Exposure to colour developing agents was not associated with the onset of lichen planus in any case. Massive exposure to the black-and-white developer Metol had probably caused lichen planus in one case. Metol has been reported previously to have caused lichen planus in one case (Roed-Petersen & Menné, 1976). Patch testing with colour developing agents is often, but not always, positive when they have provoked lichenoid reactions (Buckley, 1958; Lidén, 1984). Similarly the negative patch test with Metol does not exclude a relationship.

The stated aggravation by dark textiles and rubber objects was not clear cut and does not confirm aggravation of lichen planus by contact with PPDA or IPPD. The cases with onset of lichen planus some time after accidental exposure to stong chemicals may have been caused by the Koebner phenomenon.

The positive reactions to PPDA after 2 weeks at the first test and at re-testing 72 h after application in Case 2 suggest sensitization from the first patch test. The very weak reactions in a few cases were not suggestive of contact allergy or provocation of lichen planus. Knudsen (1964) carried out challenge tests in six lichen planus patients with a 25% solution of CD-2 with negative results. These results support the view that there must be a process of sensitization and that lichen planus or lichenoid reaction from the colour developers is not merely a Koebner phenomenon in lichen planus-susceptible subjects. This question will be explored further in an animal model. No occupational risk group for lichen planus was identified.

The frequency of involvement of the nails was greater in this study than the 10% given by Samman (1972, 1979). This might be explained by the fact that only subjects with involvement of the extremities were included.

We conclude that, in an out-patient population with lichen planus and lesions predominantly on the extremities, very few are caused by colour developing agents, PPDA or IPPD. A few cases might be caused by other chemicals, perhaps due to the Koebner phenomenon. Patch testing with PPDA, colour developing agents and related substances is only indicated when there is a history of exposure.

## ACKNOWLEDGMENT

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# Nickel dermatitis: the reaction to oral nickel challenge

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## SUMMARY

Pompholyx in nickel-sensitive subjects can be induced by orally-administered nickel, but only by a high dose. Ingestion of 5·6 mg nickel consistently worsened the pompholyx, often with a mild toxic erythema or patch test site flare, but lower doses of nickel failed to excite reactions more frequently than did a placebo, in a double-blind study. The metabolism of nickel is complex and there was considerable variation between three normal individuals in their absorption and excretion of an orally-administered nickel load. Patients may show similar variability which could be of importance clinically, although in only one of five subjects with acute eczema was the serum nickel raised, and then only marginally. The exact role of dietary nickel in perpetuating the hand dermatitis of sensitive subjects remains unclear.

The relationship between nickel and hand dermatitis has been a source of confusion to dermatologists for three decades, ever since Calnan (1956) reported a high incidence of hand dermatitis in nickel-sensitive subjects. Christensen and Möller (1975) identified a sub-group of these patients, mostly women with microvesicular hand dermatitis, in whom simple contact with nickel-containing objects could not explain the distribution or fluctuating course of the disorder. This observation led them to speculate that systemically-absorbed nickel could perpetuate the eruption.

Nickel is an essential trace element for animal nutrition, and rats deprived of it show retarded growth and low haemoglobin levels (Schnegg & Kirchgessner, 1976). Its exact role in humans is unclear. The average daily intake of nickel has been estimated to be between 300 $\mu$g and 600 $\mu$g (Schroeder, Balassa & Tipton, 1962), although a more recent study using more refined techniques gave the lower figure of 168 $\mu$g as the mean for nine average diets (Myron *et al.*, 1978). Nickel is found in many foods but common items with a high content include whole wheat, rye, oats, cocoa, tea, gelatin, baking powder, kippered herrings, soya, red kidney beans and peas (Schroeder, Balassa & Tipton, 1962; Schlettwein-Gsell & Mommsen-Straub, 1971).

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Cooking food in stainless steel utensils can increase the nickel content but only if the food contains natural acids (Christensen & Möller, 1978); canned food may have a higher nickel content than the fresh equivalent (Brun, 1979). Most of the ingested nickel is excreted in the faeces but small quantities are eliminated in urine (Horak & Sunderman, 1973).

Christensen and Möller (1975) were the first to examine the role of dietary nickel in hand dermatitis. In their double-blind study a dose of 5·6 mg of elemental nickel produced an exacerbation in nine out of 12 subjects, with none reacting to the placebo. Kaaber, Veien and Tjell (1978) gave 2·5 mg of nickel on a single-blind basis and found that 17 out of 28 patients developed a reaction, with none responding to the placebo which was always given first. In an open study using 2·5 mg, 1·25 mg, or 0·6 mg of elemental nickel, Cronin, Di Michiel and Brown (1980) found that hand eczema worsened in all five subjects given the highest dose, in three out of five given the intermediate dose and in two out of five who took the lowest dose. However, these findings were not confirmed by the double-blind studies of Jordan and King (1979) and Burrows, Creswell and Merrett (1981). Jordan and King (1979) used a dose of 0·5 mg nickel taken on two consecutive days, whilst Burrows *et al.* (1981) gave 2 mg and then 4 mg on two successive days. In neither study did reactions to nickel exceed those to placebo, although one of Jordan and King's subjects reacted consistently to nickel challenge.

In our study we have attempted to resolve the apparent discrepancies between these previous reports.

## METHODS

We carried out a randomized double-blind crossover study using three different doses of elemental nickel, 0·4 mg, 2·5 mg and 5·6 mg, given in the form of $NiSO_4 \cdot 7H_2O$. Twenty-six subjects were studied (24 women and 2 men aged from 19 to 67 years, mean 39 years). All subjects had a history of pompholyx and a reaction to jewellery, and were positive on patch testing to 5% nickel sulphate in petrolatum. No individual was included more than once.

The first investigation in 10 subjects was with 0·4 mg nickel. Either the nickel salt in lactose, or the placebo (lactose only) in identical-looking capsules, was taken before breakfast on two successive days; the following week the complementary set of capsules was taken. Subjects were seen 3 days after each challenge, and the response was assessed. A positive response was defined as accentuation of previously noted physical signs (usually worsening of microvesicular hand eczema), or the development of new physical signs, such as eczematous or erythematous eruptions. Itching alone was not deemed to be a positive reaction.

In the second study we gave a dose of 2·5 mg elemental nickel to 10 subjects, according to the above protocol. However, the third study followed Christensen and Möller's original design by giving a single dose of 5·6 mg elemental nickel in a double-blind challenge, in this case to six subjects.

In the second and third investigations, blood was taken at each visit for serum nickel estimation. Blood was withdrawn initially through plastic intravenous cannulae but this was found to be an unnecessary precaution, and samples were subsequently taken using a regular size 21 G needle. The blood was placed in acid-washed tubes and centrifuged and the serum was stored in acid-washed tubes at 4°C prior to analysis.

Nickel levels were estimated by electrothermal atomic absorption spectrometry. This technique had a sensitivity in serum of down to 0·1 μg/l of nickel and the recovery of nickel from serum or urine was quantitative. Serum samples, diluted 1 in 3 with 0·25% Triton X-100, and urine samples, undiluted, were analysed directly against aqueous nickel standards.

Contamination from the use of needles and syringes was up to 0·5 μg/l of nickel; no contamination from the urine containers was evident.

In order to study the metabolism of nickel, we investigated the effect of a single oral dose of 2·5 mg elemental nickel in three normal males (mean age 36 years), all of whom gave no history of jewellery dermatitis. None of the subjects demonstrated any cutaneous reaction during the study. Blood was taken before, and at 3, 24 and 48 h after, challenge. Urine collections were made over the 24 h prior to challenge, and over the subsequent two 24 h periods. Serum nickel was also estimated in five nickel-sensitive patients with active hand dermatitis.

An assessment of dietary nickel was carried out for six subjects. Unfortunately, the average daily nickel intake could not be quantified, although none of the individuals ate excessive amounts of foods with a high nickel content.

## RESULTS

Responses to the three different doses of oral nickel and to the placebo are shown in Table 1. For the 0·4 mg and 2·5 mg nickel doses nickel-induced reactions did not exceed those following placebo. Indeed, one of the problems in assessing this type of hand dermatitis is its tendency to fluctuate from week to week. However, following 5·6 mg nickel, all six subjects either developed pompholyx or had an exacerbation of pre-existing microvesicular hand dermatitis. Only two showed similar changes following placebo.

TABLE 1. Responses to oral challenge with nickel and placebo in nickel-sensitive subjects

| | 0·4 mg nickel (2 doses) | 2·5 mg nickel (2 doses) | 5 6 mg nickel (1 dose) |
|---|---|---|---|
| Reaction after nickel and placebo | 2 | 3 | 2 |
| Reaction after nickel only | 3 | 2 | 4 |
| Reaction after placebo only | 2 | 1 | 0 |
| No reaction | 3 | 4 | 0 |
| Total | 10 | 10 | 6 |

In addition to hand dermatitis of increased severity, other types of reaction were encountered (Table 2). Some subjects experienced eczema at areas of previous involvement, including the sites of jewellery reactions. Itching of the palms without an eruption was noted in four subjects, but this was not counted as a positive reaction because objective change was lacking. Generalized itching occurred in three subjects, but was associated with local eczematous reactions in two cases. The development of a mild morbilliform toxic erythema on the limbs, trunk and face in three of the six subjects given 5·6 mg nickel, was of particular interest. This eruption, which appeared within 12 hours and lasted for between 2 and 4 days, also occurred in one subject after 0·4 mg nickel and in another following placebo. Three of those who received 5·6 mg nickel also noticed a flare-up at their patch test site. All of the reactions developed within 48 h of challenge, the majority within the first 24 h.

The mean basal serum nickel level in the subjects with quiescent dermatitis prior to challenge was near to the accepted normal level of 1·0 μg/l or less (Table 3). The level 72 h post-placebo

TABLE 2. Reaction patterns after oral nickel challenge in nickel-sensitive subjects

| | No. of subjects | | | |
|---|---|---|---|---|
| Reaction pattern | Placebo (*n*=26) | 0·4 mg nickel (*n*=10) | 2·5 mg nickel (*n*=10) | 5·6 mg nickel (*n*=6) |
| Pompholyx worse | 7 | 3 | 3 | 6 |
| Red itchy palms | 0 | 0 | 1 | 0 |
| Patch test flare | 0 | 0 | 0 | 3 |
| Jewellery flare | 2 | 2 | 1 | 2 |
| Toxic erythema | 1 | 1 | 0 | 3 |
| No reaction | 16 | 5 | 5 | 0 |

TABLE 3. Serum nickel levels in nickel-sensitive subjects

| Serum nickel μg/l (mean ± SEM) | | | | |
|---|---|---|---|---|
| Active dermatitis (*n*=5) | Pre-challenge (dermatitis quiescent) (*n*=15) | After placebo (*n*=15) | 72 h after 2·5 mg Ni (2nd dose) (*n*=8) | 72 h after 5·6 mg Ni (*n*=5) |
| 0·8±0·2 | 1·2±0·3 | 1·0±0·2 | 2·7±0·2 | 2·6±0·7 |

was similar. Seventy-two hours after the single 5·6 mg dose or the second 2·5 mg dose of nickel, the mean serum nickel concentrations were about two and a half times the basal level. The serum nickel level was elevated above the basal level in every subject following nickel challenge, thus confirming compliance. Occasional problems were encountered with the serum nickel assay, a few high 'rogue' values being encountered. It is presumed that these resulted from unidentified contamination.

Results of challenge with 2·5 mg nickel in three normal individuals are shown in Figure 1. All

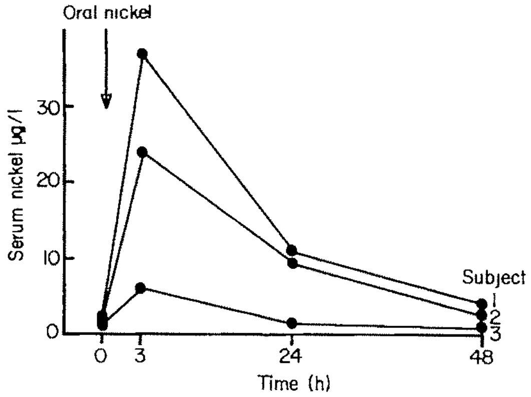

FIGURE 1. Serum nickel levels following a single oral dose of 2·5 mg elemental nickel in three normal males, aged 26 years (Subject 1), 38 years (Subject 2), and 45 years (Subject 3).

TABLE 4. 24 h urinary nickel excretion in three normal subjects; basal levels and levels after 2·5 mg of nickel orally

| Subject | Age (years) | 24 h pre-challenge (μg) | 1st 24 h post-challenge (μg) | 2nd 24 h post-challenge (μg) |
|---|---|---|---|---|
| 1 | 26 | 4·0 | 43·4 | 46·5 |
| 2 | 38 | 5·2 | 24·0 | 5·3 |
| 3 | 45 | 0·4 | 29·9 | 11·4 |

showed increases in serum nickel levels at 3 h but the magnitude of the increases varied from 30 times normal to only a 5-fold increase. Levels fell subsequently but were still elevated above the normal value in all three subjects at 48 h. The peak serum level was inversely related to the age of the subjects, the oldest subject having the lowest value. The 24 h urinary nickel excretion (Table 4) did not exactly mirror the serum values. Subject 1, who had the highest peak serum level, excreted the largest amount of nickel in the urine (over 10 times his control value during each of the two post-challenge 24 h periods), whereas Subject 3, who had the lowest serum levels, excreted larger amounts of nickel in the urine than did Subject 2. Hence, there is considerable variability between individuals in the amount and rate of absorption and excretion of nickel.

The mean serum nickel level in five females with active hand dermatitis was near to the norm (Table 3) although one individual had a slightly raised value at 1·57 μg/l.

## DISCUSSION

Pompholyx in nickel-sensitive subjects can be induced by oral nickel, specifically by 5·6 mg of elemental nickel. Our data for doses of nickel lower than this did not reveal any excess of reactions to nickel over those following placebo. In other words, it seems that the pompholyx response is a dose-dependent phenomenon. These findings help to explain some of the differences between previous investigations, and re-emphasize the need for double-blind studies.

How might the systemically-induced nickel reaction be mediated? Polak and Turk (1968) investigated the eczematous flare at previous contact sites and the generalized erythema which occurred in sensitized animals systemically challenged with chromium. In their study, the generalized reaction occurred only when a dose of a particular magnitude was given to highly sensitive animals. They concluded that, in view of the speed of onset of the reactions, circulating immune complex mediated immunity similar to the Arthus reaction (Gell and Coombs Type III hypersensitivity) was the most likely explanation of events. However, Christensen *et al.* (1981), studying orally-induced patch test site flare-ups in nickel-sensitive humans, found a dermatitis-like picture on histology. They considered that cell-mediated (Type IV) hypersensitivity was more likely to account for this type of eczematous flare-up, although humoral-mediated immunity might explain the toxic erythematous reaction which they did not investigate. Alternative views are that this toxic erythema may represent a form of Jones-Mote reaction (cutaneous basophil hypersensitivity), or that these phenomena are non-immunological, and are the result of a chemical effect of the systemically-absorbed nickel.

An intake of 5·6 mg of elemental nickel is unlikely to be encountered in the normal diet and is at least 10 times the usual daily intake. It therefore seems unlikely that systemically-absorbed nickel can play a role in perpetuating the eruption. However, before dismissing this completely, it must be noted that the absorption and metabolism of nickel varies considerably between individuals, and a handful of subjects did seem to respond to nickel at the lower doses. Hence, further investigations will need to monitor carefully the immediate nickel absorption and excretion in nickel-sensitive subjects; although we know that serum levels are not raised in patients with an active dermatitis, their status before exacerbations occur is not known.

## ACKNOWLEDGMENTS

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# Electrical field stimulation of isolated primate sweat glands

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## SUMMARY

An *in vitro* method is described for inducing secretion in isolated monkey eccrine sweat glands by means of electrical field stimulation (EFS). Ultrastructural analyses of these glands revealed unmyelinated nerve fibres surrounding the secretory coils. Numerous small clear vesicles, characteristic of cholinergic nerve terminals, were observed, along with a few which were large and dense-cored. EFS elicited an immediate secretory response which ceased abruptly upon termination of the current. The response was inhibited in a dose-dependent manner by atropine (IC50 $1{\cdot}5 \times 10^{-9}$ mol/l). Although most glands were completely inhibited by atropine, a minor atropine insensitive component was operative in some preparations. Physostigmine ($10^{-6}$ mol/l) potentiated the response to subthreshold EFS. Lidocaine ($3 \times 10^{-4}$ mol/l) completely and reversibly blocked EFS but had no effect on methacholine ($10^{-6}$ mol/l) induced secretion. These results confirm that eccrine sweat gland activation is predominantly via cholinergic pathways and that EFS of isolated glands may be a useful model to study the control of secretory function in normal and diseased states.

Pharmacological studies to elucidate the regulatory mechanisms of sweating have been conducted both *in vivo* (Foster & Weiner, 1970; Clubley *et al*., 1978) and *in vitro* (for review *see* Sato & Sato, 1981; Sato, 1984). *In vivo* methods are complicated by systemic effects as well as by numerous local factors which are not well controlled, such as skin temperature, blood circulation, oxygen supply and periglandular drug concentration. *In vitro* methods, although technically demanding and tedious, afford the advantages of longer experimental times, greater control and ease of manipulation of experimental variables. To date, however, the use of isolated glands or skin punches has been confined to the examination of events which occur after receptor activation following the exposure of the gland to chemical stimuli (Quinton, 1981; Sato, 1984).

A technique which has been used to study the neuronal control mechanisms of other systems *in vitro*, such as the salivary glands (Nishiyama *et al*., 1980) and lacrimal glands (Wikberg, 1979) and the exocrine pancreas (Davison, Pearson & Petersen, 1980) is electrical field stimulation

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(EFS). Electrophysiological experiments performed on sweat glands *in vivo* (Wilkins, Newman & Doupe, 1938; Bickford, 1938) demonstrated that an interrupted faradic current could stimulate secretion, provided the postganglionic system was intact. This report describes the adaptation of an electrical field stimulation technique to tissue preparations containing an isolated eccrine sweat gland and its surrounding intact nerves. The technique may be used to further our understanding of the regulatory mechanisms controlling sweat secretion.

## METHODS

Eccrine sweat glands were isolated from the palms of adult female rhesus monkeys (*M. mulatta*) by a modification of the method described by Sato and Sato (1981). The animals were tranquilized mildly with Vetalar® (ketamine hydrochloride, 5–10 mg/kg, Parke-Davis, Morris Plains, NJ, U.S.A.), and 2 mm punch biopsies were taken, washed with cold (4–10°C) Hank's balanced salt solution (Gibco, Grand Island, NY, U.S.A.), and held in the cold solution until used. Experiments were conducted within 8 h of the time of biopsy.

All further handling of the excised tissue was done in a temperature-controlled tissue bath containing modified Krebs-Ringer-bicarbonate solution (115 mmol/l NaCl, 5 mmol/l KCl, 25 mmol/l $NaHCO_3$, 1·2 mmol/l $NaH_2PO_4$, 1·2 mmol/l $MgSO_4$, 10 mmol/l sodium acetate, 1 mmol/l $CaCl_2$ and 5·5 mmol/l dextrose) gassed with a 95% $O_2$:5% $CO_2$ mixture to maintain a pH of 7·45. Experiments were performed at either 32° or 37°C as indicated.

Isolation of the individual glands was performed using a stereomicroscope at 35 × magnification. Fat and excess connective tissue were teased away gently from the reabsorptive duct. The secretory coil was left embedded within the fatty matrix. The open end of the isolated duct was then drawn into the tip of a constriction pipette filled with water-saturated silicone oil (Dow Corning 225 silicone oil, Dow Corning, Midland, MI, U.S.A.). The tissue-glass junction was sealed with Sylgard 184 (Dow Corning). The glands were allowed to remain undisturbed in the bath for at least 10 min prior to the start of the experiment to check for leakage.

Sweat secreted into the pipette beneath the oil layer was collected at intervals of 5 or 10 min into constant bore capillary tubes (Frederich & Dimmock, Millville, NJ, U.S.A.). Collections were made between oil blocks to prevent evaporation. Volumes were calculated by measuring the length of the collection and applying the formula for the volume of a cylinder. Volumes were converted into the average sweat rate for the collection interval and the data expressed as nanolitres/minute.

Chemical stimulation was performed by adding appropriate amounts of stock solutions to the bath or by total exchange of the bathing medium. Washing of the tissue following various treatments was achieved by repetitively (at least three times) aspirating the contents of the bath and replacing it with warm, freshly oxygenated buffer. Glands were used in the study only if they exhibited a stable secretory response to $10^{-6}$ mol/l methacholine.

Electrical field stimulation was induced using Grass stimulators, models S9 or S44 (Quincy, MA, U.S.A.). Two platinum alloy needle electrodes were placed on opposite sides of the gland at a distance of 150–200 μm from the tissue. Square (monophasic) or biphasic pulses of 5 millisecond (ms) duration were used. In preliminary experiments this pulse duration was found to be optimally effective over a range of currents and frequencies. No differences were noted in tissue responsiveness between the two pulse types; however, the latter minimized the formation of gas bubbles and electrolytic deposition on the electrodes during prolonged stimulation.

For electron microscopy, biopsies were immediately fixed for 2 h with Karnovsky's fixative (Karnovsky, 1965) diluted to one-half strength with distilled water. A few glands were dissected from the biopsy as previously described, for a comparison of the morphology with that of intact tissue. The specimen was then placed in 0·3 mol/l buffered sucrose prior to fixation. The tissue was postfixed with $OsO_4$, stained with uranyl acetate and embedded with epoxy resin by the technique of Tandler and Walter (1977). Appropriate ultrathin sections were stained with lead citrate before examination using a Siemens Elmiskop 101 electron microscope.

## RESULTS

### *Electron microscopy*

Unmyelinated nerve fibres appeared in a loose sleeve-like formation outside the basement membrane surrounding the secretory coils of the eccrine glands. Occasional varicosities characterized by abundant synaptic vesicles and clusters of mitochondria were noted (Fig. 1).

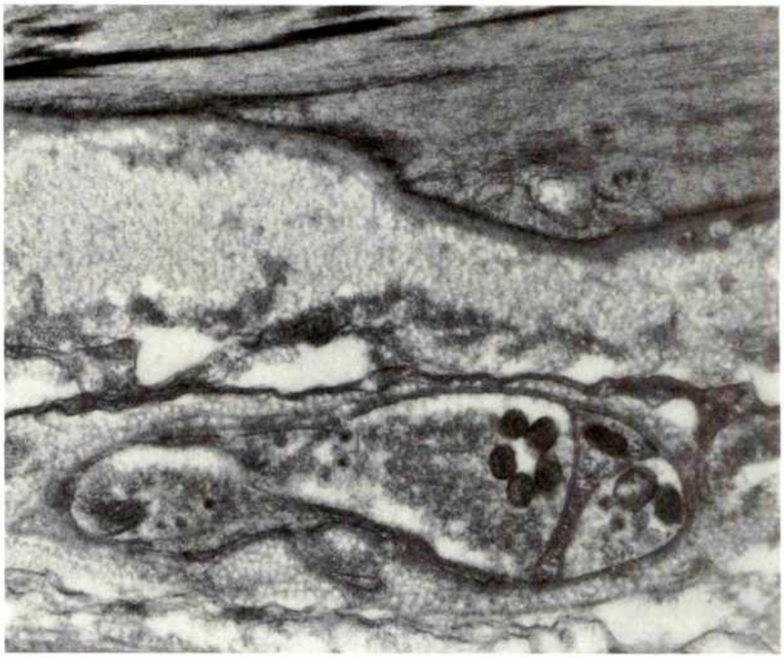

FIGURE 1. Electron micrograph of a cluster of nerve terminals in close relation to a myoepithelial cell (top) on the secretory coil of an eccrine sweat gland. The terminals are filled with clear synaptic vesicles, but a few of the dense-cored variety are present in each terminal (original × 24 400).

The vesicles were predominantly small and clear, typical of cholinergic nerve endings. A few large, dense-cored vesicles were observed, but selective fixation or radiolabelling would have been necessary to establish them definitively as adrenergic. Varicosities interior to the basement membrane of the secretory coil or in direct contact with glandular cells were not observed. Ultrastructural comparison of individual glands and biopsy specimens confirmed that the dissection procedure did not damage the surrounding nerve terminals and that the glands were suitable for electrical stimulation experiments.

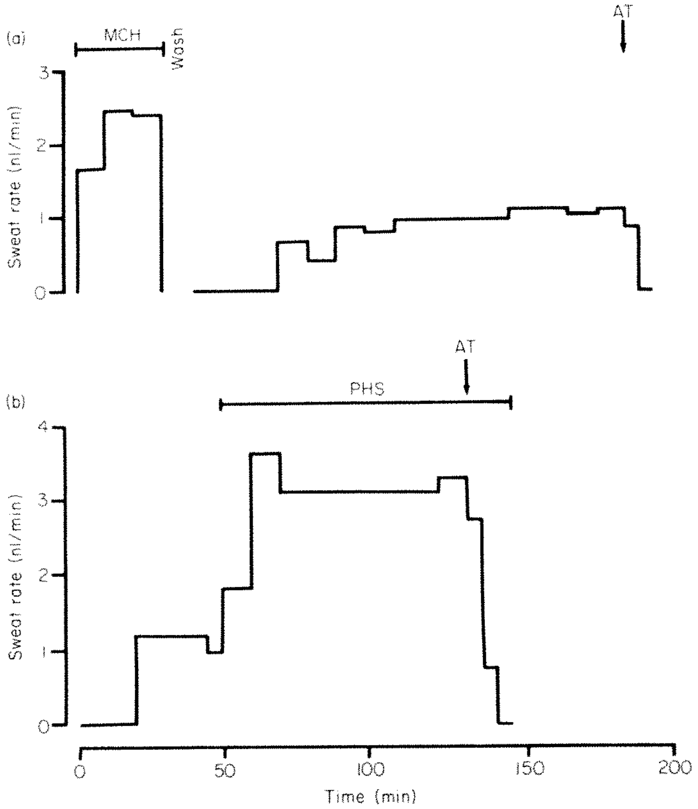

FIGURE 2. Spontaneous secretion in two glands at 37°C. (a) The magnitude of spontaneous secretion is compared with that induced by methacholine (MCH, $10^{-6}$ mol/l). The delayed onset after medium exchange and inhibition by atropine (AT, $10^{-7}$ mol/l) are shown; (b) augmentation of the spontaneous secretion by non-agonist concentrations of physostigmine (PHS, $10^{-6}$ mol/l) and inhibition by AT.

### *Spontaneous secretion*

At 37°C most glands secreted spontaneously within 5–20 min after being drawn up into the constriction pipette. The magnitude of the secretion over time approached that produced by a maximally effective concentration of methacholine ($10^{-6}$ mol/l). Extensive exchange of the bathing medium delayed the onset of this secretion but did not eliminate it. Spontaneous secretion was inhibited by atropine ($10^{-7}$ mol/l) and augmented by non-agonist concentrations of physostigmine ($10^{-6}$ mol/l) (Fig. 2). Thus, the phenomenon is probably a result of leakage of acetylcholine from adjacent nerve endings. Reduction of the tissue bath temperature to 32°C eliminated spontaneous secretion while maintaining tissue responsiveness to pharmacological stimuli. Since spontaneous secretion was quite irregular over the course of an experiment, this manipulation was employed in subsequent experiments to provide a stable background on which the effects of chemical and electrical stimulation could be determined.

### *Electrical stimulation*

Tissue responsiveness to increasing electrical current was determined using pulses of 5 milliseconds (ms) duration at a frequency of 5 pulses/second (Hz). A steep response curve was

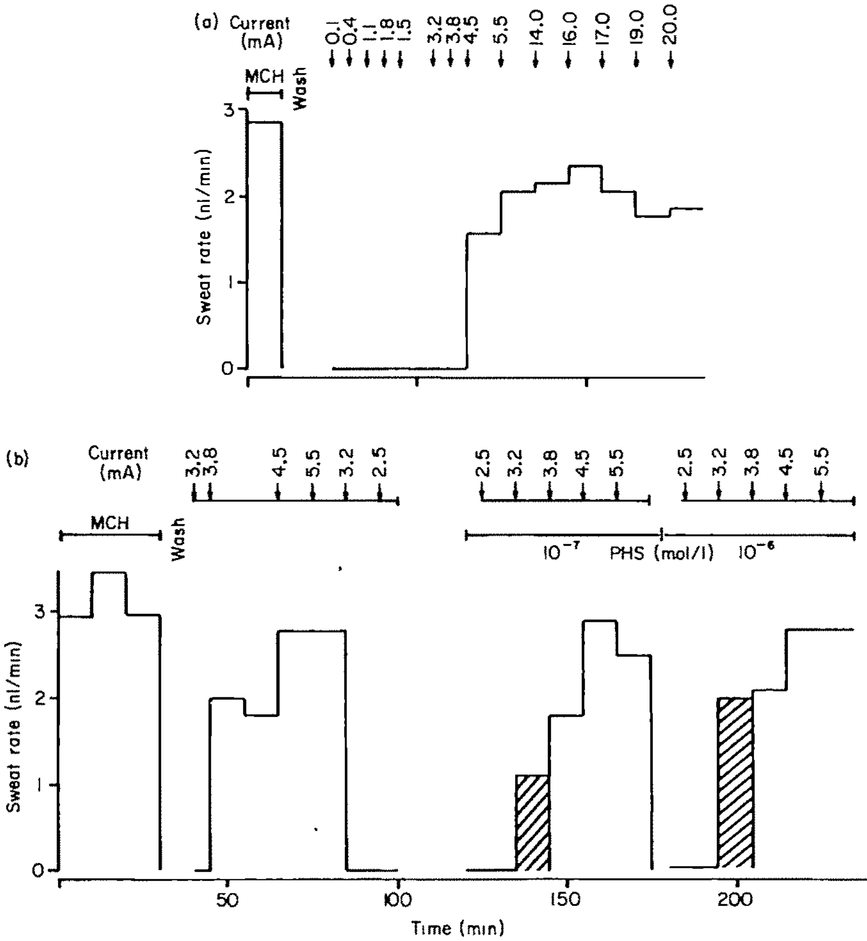

FIGURE 3. Electrically-induced secretion in two glands at 32°C. (a) Response to increasing current relative to the MCH ($10^{-6}$ mol/l) induced response; (b) physostigmine (PHS) augmentation of response (5 ms pulses, 5 Hz). ▨ = secretion at current 3·2 mA which was subthreshold in the absence of PHS.

obtained by increasing the current in 1–2 milliampere (mA) increments. The initial response to a threshold current was 60–100% of that produced by a maximally effective current (Fig. 3a). The addition of non-agonist concentrations of physostigmine ($10^{-7}$–$10^{-6}$ mol/l) to glands subjected to currents 0·6–1·0 mA below the threshold resulted in secretion (Fig. 3b), evidence that acetylcholine is released from the nerve endings in subthreshold concentrations in response to electrical stimulation. The responses to higher currents remained unchanged in the presence of physostigmine.

Ninety-five percent of the glands tested achieved maximum electrically-induced secretory rates with 5·5 mA and 100% did so with 7·5 mA (Fig. 4a). With the current fixed at either of these levels, the effect of various pulse frequencies was determined. As depicted in Figure 4b, the initial responses at 5 and 10 Hz were significantly greater than at 1 Hz ($P = 0{\cdot}01$ and 0·05, respectively, Student's $t$-test) while the average response over 2 h at 5 Hz was significantly greater than at either 1 or 10 Hz ($P = 0{\cdot}05$). A loss of responsiveness to EFS at 10 Hz over time

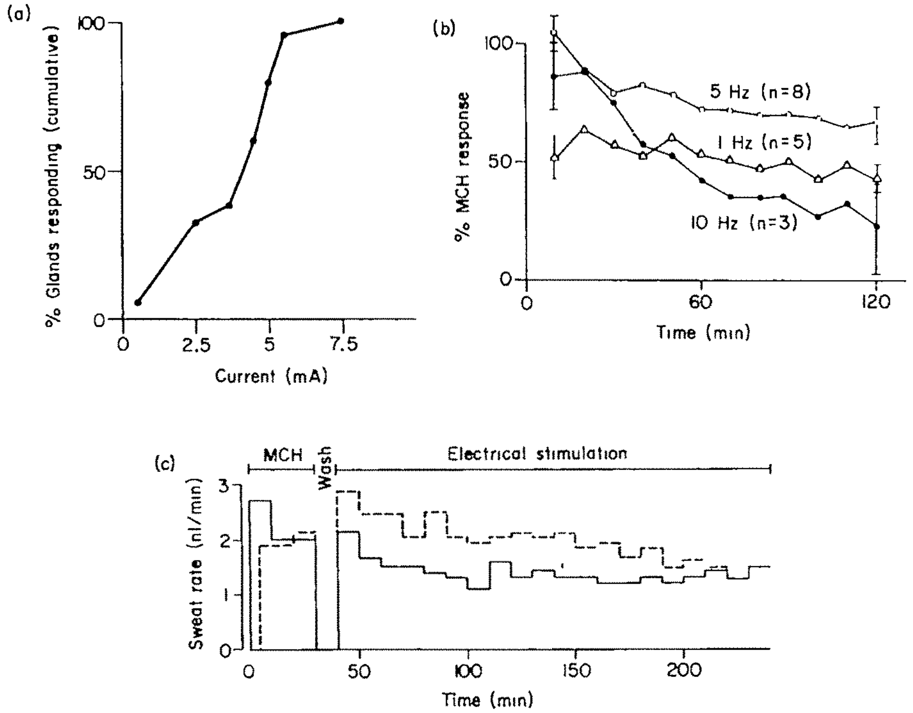

FIGURE 4. Optimal conditions for electrical stimulation (a) Cumulative percentage of glands stimulated at various currents ($n = 20$); (b) average responses over time to various stimulation frequencies (5·5 or 7·5 mA, 5 ms, 32°C). Data are expressed relative to a maximally effective concentration of MCH ($10^{-6}$ mol/l). Points are means ± SEM; (c) typical responses of two glands to optimum parameters. ——— 5·5 mA, 5 ms, 5 Hz; —— 7·5 mA, 5 ms, 5 Hz.

suggested the depletion of available transmitter rather than 'fatigue' of the gland since responsiveness to methacholine was maintained.

Based on the results described above, the conditions selected as optimum for inducing a maximal stable and prolonged secretion were 5·5 or 7·5 mA current pulses of 5 ms duration at 5 Hz. Characteristic responses using these parameters are presented in Figure 4c. The average response was stable for 2 h of constant stimulation, although some glands continued to secrete at a constant rate for longer than 3 h. The response to electrical stimulation was immediate and ceased abruptly upon termination of the stimuli. Employing 10 min collection intervals, the coefficient of variation (standard deviation expressed as a percentage of the mean response) of the electrically-induced response was comparable to that of methacholine-induced ($10^{-6}$ mol/l) secretion (17% and 16%, respectively).

Lidocaine ($3 \times 10^{-4}$ mol/l) completely inhibited electrically stimulated secretion while having no effect on the secretion induced by methacholine. The inhibition produced by lidocaine was reversed at least partially by removal of lidocaine from the bathing medium (Fig. 5).

Atropine ($10^{-8}$ mol/l) completely inhibited electrically-induced secretion in approximately 70% of the glands evaluated. The inhibition was dose dependent, yielding an IC50 value of $1{\cdot}5 \times 10^{-9}$ mol/l (Fig. 6). The remaining 30% of the glands continued to secrete, at a rate

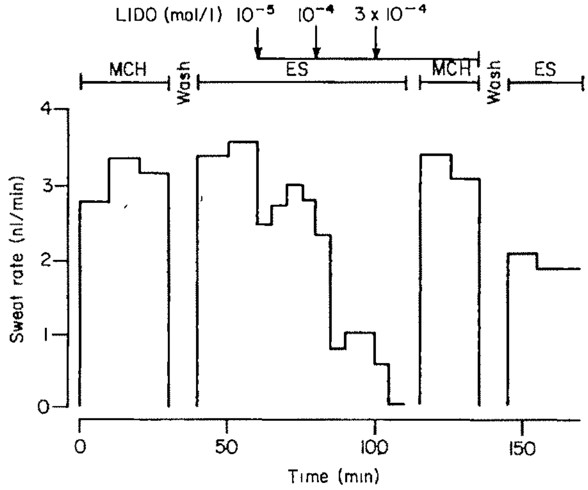

FIGURE 5. The effect of lidocaine (LIDO) on secretion induced by electrical stimulation (ES) and methacholine (MCH, $10^{-6}$ mol/l).

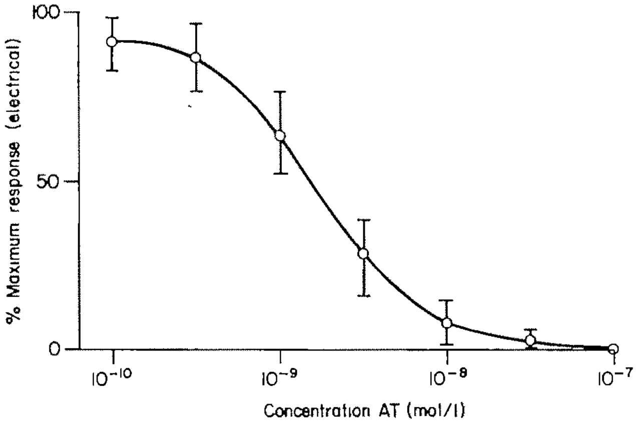

FIGURE 6. Inhibition of electrically-stimulated secretion by atropine. Points represent means of 6 determinations ± SEM.

approximately 15% of maximum, in the presence of $10^{-7}$–$10^{-6}$ mol/l atropine, suggesting that a minor atropine-insensitive component may be operative in some preparations.

## DISCUSSION

The use of electrical field stimulation (EFS) to study the neural control of eccrine sweat glands *in vivo* was first described by Wilkins *et al.* (1938) and Bickford (1938). Application of the technique to *in vitro* studies relies on: (a) the presence of numerous nerve fibres surrounding the gland (Montagna & Parakaal, 1974; Uno & Montagna, 1975; Uno, 1977), (b) preservation of that innervation during isolation of the gland (shown in the present study), and (c) responsiveness of the isolated gland to chemical stimuli as demonstrated by the comprehensive studies of Sato (1984). Depolarization of intact nerve endings results presumably in release or synthesis of endogenous neurotransmitter(s), interaction of neurotransmitter(s) with receptors on the sweat gland, and activation of a sequence of biochemical events culminating in fluid secretion into the lumen of the sweat duct. *In vitro* EFS affords the opportunity to examine this sequence of events under well controlled conditions and in the absence of complicating factors which are operative *in vivo*. Under the conditions described, glands can be maintained for up to 8 h following biopsy and can be induced to secrete uniformly for 2 h or more. The magnitude of EFS-induced secretion was comparable to that of a maximally effective concentration of methacholine. Interestingly, the concentration of atropine which produced a 50% inhibition of EFS (−log molar concentration, 8·8) corresponded to that reported by Sato and Sato (1981) for the inhibition of methacholine-induced secretion ($pA_2$, 8·9).

Primate sweat glands possess both adrenergic and cholinergic innervation (Uno & Montagna, 1975). A predominant role for the cholinergic system is suggested by: (a) the ability of atropine and other muscarinic receptor blocking drugs to inhibit thermal and emotional sweating *in vivo* (Sato, 1977), (b) the greater potency of cholinergic versus adrenergic agonists as secretogogues both *in vivo* (Foster, Ginsburg & Weiner, 1970) and *in vitro* (Sato & Sato, 1981), and (c) histochemical and ultrastructural analysis showing nerve terminals which are mostly cholinergic (Uno, 1977). The present experiments with EFS confirm that neuronal activation of the eccrine sweat gland is predominantly via cholinergic pathways. Indeed, the effects demonstrated for physostigmine indicate that acetylcholine is released even at subthreshold currents. Considering that these glands are responsive to adrenergic agonists (Sato & Sato, 1981), it is possible that adrenergic receptor activation *in vivo* may occur primarily via humoral rather than neuronal pathways. Incomplete inhibition by atropine in approximately 30% of the glands tested suggests the possible involvement of noncholinergic mediators. Additional studies using EFS of isolated glands are needed to investigate whether other neurotransmitters also play a physiological role in gland activation.

## ACKNOWLEDGMENTS

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# Gianotti-Crosti syndrome: a study of 26 cases

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## SUMMARY

We have studied 26 patients presenting with a symmetrical papular or papulovesicular acrolocated eruption of more than 10 days duration. Mean age at onset was 2 years (range 10 months to 5·75 years). Lymphadenopathy was noted in eight cases, and hepatomegaly in one case. In 12 cases, histopathology and direct immunofluorescence were non-contributory. Cytolytic hepatitis occurred in one case and was associated with HBs antigenemia. A history of recent immunization was given in two cases. There was serological evidence of recent Epstein-Barr virus infection in seven out of 13 cases tested. Coxsackie B viruses were isolated from three patients, and cytomegalovirus was probably associated with the syndrome in one case. We conclude that the Gianotti-Crosti syndrome is not rare in France, and that non-hepatitis B virus (HBV)-associated cases are more frequent than the classical HBV-associated papular acrodermatitis of childhood.

Papular acrodermatitis of childhood (PAC), first described by Gianotti (1955), mainly affects children from 2 to 6 years of age. It is characterized by a triad of a papular rash, localized to the cheeks, limbs and buttocks, lymphadenopathy, and anicteric acute hepatitis. This distinctive eruption coincides with a primary infection with hepatitis B virus (HBV) (Gianotti, 1973). The virus sub-type *ayw* is the most common (Ishimaru *et al.*, 1976). In a re-appraisal of the progress made in the understanding of this disease since its discovery, Gianotti (1979) emphasized the need to separate this entity from other frequently-encountered papular or papulovesicular acrolocated eruptions of unknown origin which he classified as 'papulovesicular acrolocated syndrome' (PAC) and further sub-divided into three types (A, B and C) on the basis of subtle clinical clues. However, the distinctions between these rashes are difficult to make in practice, and since then, other aetiological agents, mainly viruses (enteroviruses, adenoviruses, parainfluenzaviruses, Epstein-Barr virus and cytomegalovirus) have been found to cause typical PAC-like rashes. Thus, it seems more appropriate to follow Rogers and Conolly (1974) and San

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Joaquin and Marks, (1982) and to apply the term 'Gianotti-Crosti Syndrome' to all the rashes with the typical pattern of PAC, and to distinguish between them further on the basis of their cause.

Few series of patients with Gianotti-Crosti syndrome (GCS) have been reported outside northern Italy, except for the British series described by Eiloart(1966), which lacks aetiological data, the epidemiologically-oriented paper by Ishimaru *et al.* (1976) which deals only with hepatitis B related cases, and the small series of non-HBV related cases from the Mayo Clinic (Spear & Winkelmann, 1984).

We report here the clinical, histological and aetiological features of 26 cases of GSC seen over a 5-year period in a paediatric dermatology department in an urban area of south western France.

## METHODS

### *Patients*

In the present study we reviewed retrospectively the records of 26 patients (17 inpatients and 9 outpatients) seen in our department between January 1980 and June 1985. The requirement for inclusion in the study was the presence of a symmetrical papular or papulovesicular rash of more than 10 days duration, affecting the limbs, cheeks and buttocks, but without, or with minimal, trunk involvement. Presence of hepatomegaly or lymphadenopathy was not considered a prerequisite for inclusion. The following details were obtained, if available: (a) history of prodromes, and previous immunizations; (b) presence of systemic constitutional symptoms; (c) presence of pruritus, type of lesions (papule, vesicle or mixed type) and their distribution (grouped or isolated). Photographic material was available for review in 18 cases; (d) associated physical findings, and (e) laboratory tests. These are listed in Table 1.

Of the 26 patients, 14 were male and 12 female. Twenty-three were Caucasians, mostly of French origin, two were black, and one of Asian origin. Mean age at onset was 2 years (range 10 months to 5·75 years). Two cases presented in 1980, four in 1981, two in 1982, two in 1983, 11 in 1984, and five in 1985. Seven cases presented in January to March, six in April to June, nine in July to September and four in October to December.

TABLE 1. Laboratory investigations carried out in 26 patients with GCS

| Test | No. of patients tested |
|---|---|
| Full blood count | 25 |
| Aminotransferases (ASAT, ALAT) | 26 |
| Histopathology | 12 |
| Direct immunofluorescence | 12 |
| HBs antigen and antibodies | 26 |
| Antibodies to: | |
| Adenovirus | 17 |
| Para-influenza virus I and III | 17 |
| Enterovirus | 17 |
| EBV | 13 |
| Coxsackie B virus | 8 |
| CMV | 7 |
| Viral cultures (throat swab and stool) | 10 |

### Histopathology

In the 12 patients from whom paraffin-embedded biopsy material was available for review, sections were stained with Haematoxylin-Eosin Safran (HES) and Periodic Acid Schiff (PAS). Primary histological lesions were assessed comparatively and histopathological features graded semi-quantitatively (−: absent, +: mild, + +: moderate, + + +: marked). Direct immunofluorescence study of involved skin was performed according to routine techniques.

### Virology

Throat swabs and stool samples were inoculated into either human diploid cells (MRC5, Bio Mérieux, Craponne, France), Vero cells (INSERM U 117) or Vervet monkey kidney cells (Institut Pasteur, Paris, France). Antibodies to adenoviruses, para-influenza viruses I and III, enteroviruses (pooled) and coxsackie B1 to B6 viruses were assayed using a standard complement fixation (CF) test.

Antibodies to cytomegalovirus (CMV) were tested for using a CF test and by enzyme-linked immunosorbent assay (ELISA) for IgG and IgM (Virion, distributed by Biochema BP3045, 3002 Nîmes Cedex, France).

Antibodies to Epstein-Barr virus (EBV) were tested for using immunofluorescence for the following markers: VCA IgM (Gull, distributed by Orimbio, Paris, France), VCA IgG (Institut Pasteur, Paris, France), EBNA IgG (Hyland, distribted by Prochilab, Bordeaux, France).

HBV markers were tested for using ELISA (Abbott, Rungis, France) for HBs antigen and antibody, HBc antibodies (IgG and IgM) and HBe antigen and antibody.

### Controls

Two age-matched patient groups (age range 6 months to 5 years) were used as controls. These patients had all been admitted to the same hospital for various illnesses, but none had had skin lesions.

*Group 1.* Sera from 34 patients, sampled at random during 1984, were tested for long-term antibodies to herpesviridae (Bonnici *et al.*, 1985) using immunofluorescence for antibodies to EBV VCA, and ELISA for antibodies to CMV.

*Group 2.* Group 2 consisted of 45 consecutive patients hospitalized between April and September 1985 for whom viral investigations were requested. This group was assessed retrospectively for evidence of infection with adenoviruses, para-influenza viruses I and III, and enteroviruses, as described above.

## RESULTS

### Clinical features

Typical clinical features are illustrated in Figure 1 and the main findings are summarized in Table 2. Prodromes occurred in more than one quarter of cases. They consisted of diarrhoea (two cases, one being associated with acute rhinitis), acute rhinitis in isolation (three cases), and tonsillitis (three cases). Fever, when present, was low grade, except in two patients who had a temperature of 39°C on admission.

Two patients had been immunized 10 days and 14 days before the onset of the eruption with BCG and with combined poliomyelitis-diphteria-tetanus-pertussis vaccine respectively.

Papules or papulovesicles were 2–5 mm in diameter. Koebner's phenomenon was noted in one case and purpura was not recorded. Coalescence of lesions was assessed using photographic data, and was noted in only four cases out of 18. In 14 patients, duration of the rash could not be

TABLE 2. Clinical features of 26 patients with GCS

| Symptoms | No. of patients (%) |
|---|---|
| Prodromes | 8 (31) |
| Fever | 7 (27) |
| Recent immunization | 2 ( 8) |
| Rash (type) | |
| Papular | 14 (54) |
| Papulovesicular | 12 (46) |
| Rash (duration) | |
| 2 weeks | 6 (23) |
| 3 weeks | 2 ( 8) |
| 4 weeks or more | 4 (15) |
| Unknown | 15 (57) |
| Pruritus | 6 (23) |
| Lymphadenopathy | 8 (31) |
| Hepatomegaly | 1 ( 4) |

assessed because they were lost to follow-up. The longest duration (16 weeks) was observed in a 3-year-old girl in whom no underlying aetiology was found.

*Laboratory investigations*

*Blood cells counts.* Hyperlymphocytosis was found in 10 cases, ranging from 5146 to 9430 cells/mm$^3$ (normal age-matched values: 3000–4900 cells/mm$^3$). Monocytosis (1500 cells/mm$^3$) was found in two cases. Turk cells were observed in six cases.

TABLE 3. Histopathological findings in 12 patients with GCS

| | Patient no. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 6 | 7 | 8 | 9 | 11 | 15 | 16 | 17 | 20 |
| Epidermis: | | | | | | | | | | | | |
| Parakeratosis | − | ++ | + | + | + | − | − | + | − | + | + | − |
| Acanthosis | + | + | − | + | + | + | − | + | − | − | + | + |
| Papillomatosis | − | − | − | + | + | + | − | − | − | − | − | + |
| Spongiosis | − | − | − | − | + | − | − | − | − | + | − | − |
| Exocytosis | + | + | + | − | ++ | ++ | − | ++ | + | + | + | + |
| Dermis: | | | | | | | | | | | | |
| Oedema | Present | − | − | − | − | Present | − | − | − | − | − | − |
| Infiltrate, superficial dermis | + | + | ++ | + | ++ | +++ | + | +++ | + | ++ | +++ | ++ |
| Infiltrate, mid and lower dermis | − | + | ++ | + | ++ | +++ | + | +++ | + | ++ | +++ | ++ |
| Subcutaneous fat: (infiltrate) | NA | − | NA | NA | NA | NA | NA | − | NA | − | +++ | − |

Grading of infiltrate: − = absent; + mild; ++ moderate; +++ marked.
NA = Material not available.

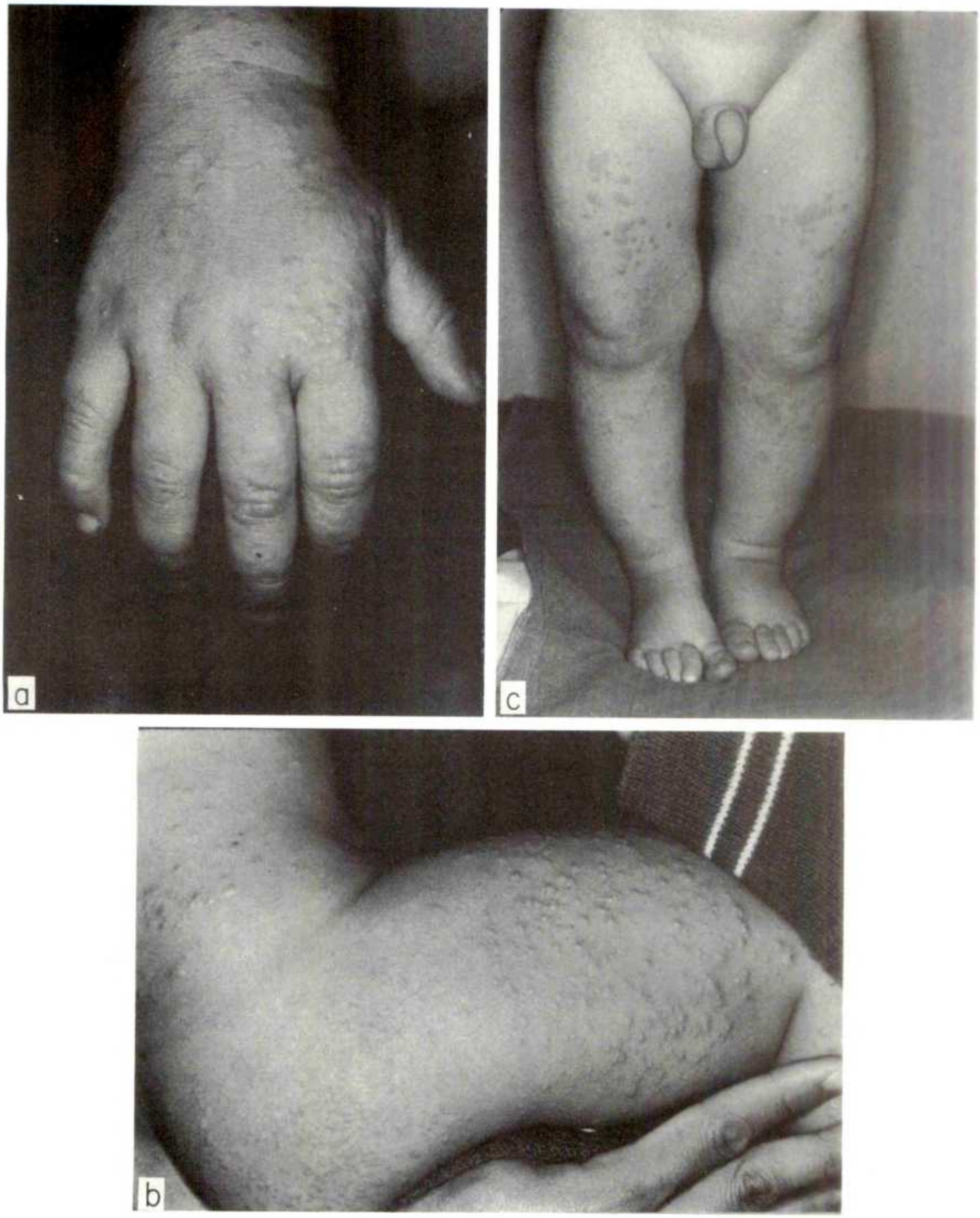

FIGURE 1. Clinical features of Gianotti-Crosti syndrome (a) papular lichenoid rash (Patient 13); (b) papulovesicular rash (Patient 1); (c) papuloedematous rash (Patient 10).

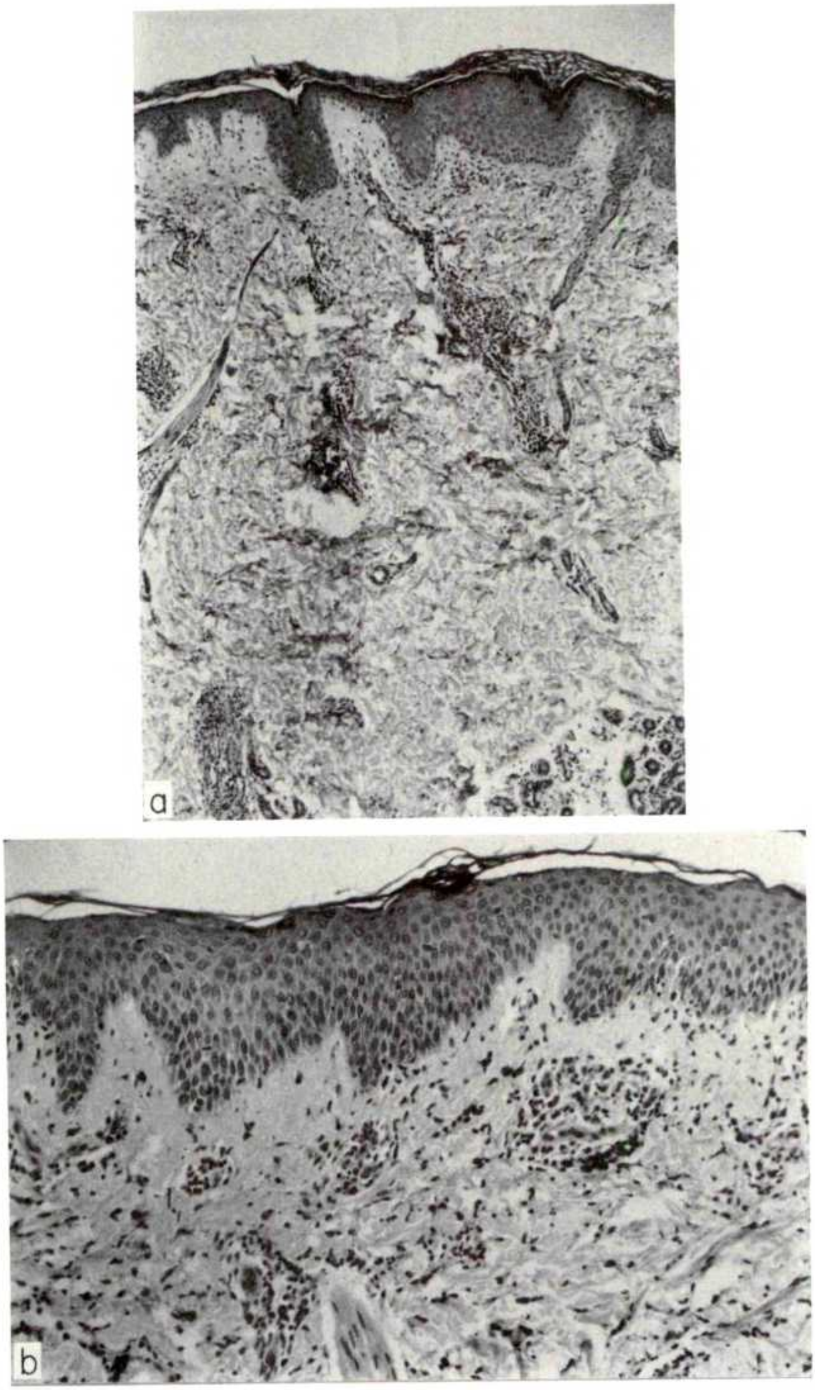

FIGURE 2. Histopathology (a) pattern of dermal infiltrate; (b) perivascular infiltration of the epidermis by mononuclear cells. Note a focus of parakeratosis and the narrowing of the capillary lumen. (Patient 16). (HES).

TABLE 4. Positive virological findings in eight patients

| Patient no. | Sex | Age at onset (months) | Year of onset | Time of year* | Rash type | Total duration | Associated findings | HBs antigen | Other viruses |
|---|---|---|---|---|---|---|---|---|---|
| 2 | M | 69 | 1980 | 4 | PV | > 4 weeks | none | absent | Coxsackievirus B4<br>rise in antibody titres from 0–1:40 |
| 4 | M | 36 | 1981 | 1 | PV | not known | Acute rhinitis<br>fever – asthenia<br>lymphocytes 5162/$mm^3$ | absent | Heterophil antibodies (PBD, 1:40) |
| 5 | F | 10 | 1981 | 1 | PV | not known | Acute rhinitis fever<br>Turk cells<br>lymphocytes 8780/$mm^3$ | absent | antibodies to CMV 1:40 |
| 21 | F | 18 | 1984 | 3 | P | not known | none | absent | Coxsackievirus B5 (throat swab and stool cultures) |
| 22 | M | 24 | 1985 | 2 | PV | not known | lymphocytes 7260/$mm^3$ | absent | EBV VCA IgM, 1:5<br>VCA IgG, 1:640<br>EBNA, 1:10 |
| 23 | M | 24 | 1985 | 2 | PV | not known | none | absent | EBV VCA IgM < 1:5<br>VCA IgG, 1:80<br>EBNA, 1:5 |
| 24 | M | 21 | 1985 | 1 | P | 12 days | ALAT: 1100 IU/ml<br>Turk cells<br>lymphocytes 9000/$mm^3$<br>monocytes 1500/$mm^3$ | present | EBV VCA IgM positive<br>VCA IgG 1:640<br>EBNA, 1:10 |
| 26 | M | 12 | 1985 | 2 | P | 10 days | Fever – acute rhinitis<br>Turk cells | absent | Coxsackie B4 throat swab and stool cultures<br>EBV: VCA IgM < 1:5<br>VCA IgG, 1: 640<br>EBNA, 1:5 |

P = papular; PV = papulo vesicular; PBD = Paul Bunnel Davidsohn.
*1 = Jan–March; 2 = May–June; 3 = July–Sept; 4 = Oct–Dec.

*Aminotransferases.* One case (Patient 24, Table 4), had increased serum levels of aminotransferases (ALAT up to 1100 IU/l).

*Histopathology.* Typical histopathological features are shown in Figure 2, and Table 3 details the results in 12 patients. Epidermal modifications graded as mild or moderate were found in 11 of the 12 cases. Dermal infiltrates consisted of lymphohistiocytes, always in a perivascular location, and rarely periadnexial (three cases). Involvement of subcutaneous fat was demonstrated in one case of the five from whom subcutaneous tissue was available. In the cutaneous vasculature, swelling of endothelia was moderate or marked in seven cases and the lumen was obliterated in four cases with 'marked' involvement. Features of leukocytoclastic vasculitis and fibrin deposits were not seen, and immune deposits were absent.

*Virology (patients).* Positive virological findings were obtained in eight patients and the results are summarized in Table 4. At the time of his rash, Patient 24 had detectable HBs antigenemia associated with anti HBc (IgM class) antibodies. Furthermore, his serological profile for EBV was consistent with a primary infection, since 2 months later antibodies to VCA (IgG) were found at a titre of 1:160 without IgM. Coxsackie B virus infection during the rash was demonstrated in Patients 2, 21 and 26. Since carriage of coxsackie B virus is frequent in June and July, the link with GCS in Patients 21 and 26 should be considered with caution. However, absence of detectable antibodies to coxsackieviruses in the serum samples from these two patients, taken 4 and 5 days after onset of the eruption respectively, suggests recent infection rather than carriage. A second serum sample was not available. In Patient 26, the EBV serological profile and the presence of Turk cells may indicate a recent infection. Patients 22 and 23 were twins. Presence of EBV anti-VCA IgM antibodies was demonstrated in one sibling (Patient 22) with the more recent eruption, and the serological profile in his twin was consistent with recent EBV infection. Antibodies to CMV (IgG class) were demonstrated in Patient 4; IgM and viruria were not tested for. However, the age of this patient (10 months) suggested a recent infection.

*Virology (controls).* In Group 1, IgG to EBV VCA was demonstrated in 44% of patients, and IgG to CMV in 24%. In Group 2, antibodies to para-influenzavirus type III, were found in one patient, antibodies to adenoviruses in four patients, antibodies to enteroviruses at low titres (between 1:20 and 1:40) in seven; but none had positive stool or throat swab cultures.

## DISCUSSION

Twenty-six patients with Gianotti-Crosti syndrome were seen in our department during a 5-year period. Our hospital is situated in the centre of an urban area with roughly half a million inhabitants. During these 5 years, 20 000 patients of under 5 years of age were seen in our outpatient clinic. Thus, GCS is not particularly rare.

Our data did not show substantial seasonal variation, although a few more cases were seen in the summer. According to Gianotti (1979) PAC is more frequent in spring and autumn, and American cases not associated with HBV were recorded mostly in the autumn and winter (Spear & Winkelmann, 1984). Incidence of GCS cases presenting at our clinic has risen during the last 18 months, in the absence of any epidemics. The cause of this is not clear.

In our series, GCS affected similar numbers of both sexes, and the ethnic origins of the patients did not differ from the general population. The clinical features were in accordance with previous descriptions (Gianotti, 1979; Spear & Winkelmann, 1984). The rash was monomorphic (papular) in 54% of cases and never petechial. Pruritus was moderate and unrelated to the aspect of the lesions (papular or papulovesicular). Prodromes were not specific.

However, they may give some aetiological clues (tonsillitis and EBV infection, diarrhoea and enterovirus infection). Recent immunization was found in two patients. The significance of this finding is questionable, since many children in that age group are immunized. Similar cases have been reported in the literature (Puissant, *et al.*, 1966; Spear & Winkelmann, 1984).

Among the associated clinical findings, lymphadenopathy was present in 31% of cases. Cervical lymph nodes were most often involved, with moderate enlargement. Hepatomegaly was rare (one patient out of 26). The only patient with cytolytic hepatitis and HBs antigenemia (Patient 24) had no detectable liver enlargement.

Differential diagnosis was not a major problem, since the pattern of distribution of the rash is quite distinctive. Some authors have included frictional lichenoid eruption (Dupré *et al.*, 1974) and in a recent review, Fox and Odom (1985) mentioned many papular conditions, namely, lichen planus, lichen nitidus, Letterer-Siwe disease, and lichenoid drug eruptions.

Our results confirm the non-specific nature of the histological changes, which consisted of capillary endothelial swelling, a lymphohistiocytic perivascular infiltrate and variable epidermal changes. We did not find epidermal microabcesses as did Rubenstein, Esterly & Fretzin (1978), which may lead to an erroneous diagnosis of mycosis fungoïdes. Cytology of the infiltrate in no case caused any confusion with Letterer-Siwe disease, as mentioned by these authors. Direct immunofluorescence studies remained negative, as has previously been reported in GCS in children (Gianotti, 1979). However, perivascular C3 deposits have been demonstrated in three adult cases studied by Claudy *et al.* (1977).

From the aetiological point of vew, the rarity of 'classical' PAC (associated with HBV) in our series was notable. Furthermore, the only case with HBs antigenemia (Patient 24) also had serological evidence of primary EBV infection. This patient is described in more detail elsewhere (Taïeb *et al.*, 1985). It has been demonstrated clearly in PAC that HBs antigen is present in the serum at the onset of the rash (Ishimaru *et al.*, 1976; Colombo *et al.*, 1977). Thus, 25 of our 26 cases represent PAC or PAC-like disease not associated with HBV infection. Among 18 of these patients, we could not find any aetiological factors except for the two aforementioned cases with immunization. In the remaining seven cases EBV, coxsackie B viruses and cytomegalovirus (CMV) were probably associated with the syndrome.

Asymptomatic primary infection with EBV is common in young children. In our control group 1, 44% of subjects between 6 months and 3 years of age had antibodies to EBV-VCA. The finding that 5 out of 13 patients tested in our series had evidence of recent infection is consistent with this prevalence. However, EBV infection has already been clearly established in GCS (Maleville *et al.*, 1975; Labbé *et al.*, 1981; Konno *et al.*, 1982; Revuz, Lassalle & Weschler, 1983; Ramelet, 1984; Spear & Winkelmann, 1984; Iosun, Santos & Gromisch, 1984). The role of EBV in Patients 24 and 26, in which HBs antigenemia and coxsackie B4 virus respectively, were found, is not clear. However, it is possible that EBV infection altered the patients' immune status, allowing a second viral infection.

Among enteroviruses, coxsackie virus A 16 (James, Odom & Hatch, 1982), echovirus type 7 (Labbé *et al.*, 1982) and type 9 (Rogers & Connolly, 1974) have already been associated with PAC-like eruptions. To this list should be added coxsackie viruses B4 and B5 which were associated with GCS in three of our patients.

Our control group 1 has shown that 24% of hospitalized children under 5 years of age had serological evidence of previous infection with CMV. CMV infection associated with GCS has already been clearly demonstrated (Berant, Naveh & Weissman, 1984); our evidence of CMV infection in Patient 5 was weaker than the observation of these authors, but the significant antibody titres to CMV were associated with clinical and laboratory features consistent with

CMV infection.

In our series, classical HBV-positive PAC was rare. EBV and enteroviruses may be more frequently associated with GCS in young children in France. However, the possibility of chronic hepatitis and even death due to HBV infection (Pouillaude *et al.*, 1975), means that minimal investigations including HBV serology and aminotransferase determinations are essential in cases of GCS.

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# Incontinentia pigmenti: eosinophil chemotactic activity of the crusted scales in the vesiculobullous stage

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## SUMMARY

To investigate the mechanisms underlying eosinophil infiltration into the epidermis in incontinentia pigmenti (IP), we studied the eosinophil chemotactic activity in extracts of the crusted scales from three patients with IP in the vesiculobullous stage. Eosinophil chemotactic activity was detected in the eluates from a Sephadex G-75 chromatography column between the vitamin $B_{12}$ and phenol red markers. The chemotactic activity was heat-stable and resistant to enzyme digestion, and recovered after ether extraction at low pH. Leukotriene B4 (LTB4) was demonstrated in the fractions with high eosinophil chemotactic activity. These findings suggest that LTB4 plays an important role in the accumulation of eosinophils within the epidermis in IP, in the vesiculobullous stage. Blood eosinophilia, however, may not be induced by the eosinophil chemotactic factors in the scales.

Incontinentia pigmenti (IP) is a multi-system hereditary disorder with dermatological, neurological, skeletal, ocular, and dental defects (Carney, 1976). The characteristic lesions are cutaneous, in most cases developing in three stages: vesiculobullous, verrucous, and pigmentary. The first stage is characterized by the presence of numerous eosinophils within the epidermis as well as around the epidermal vesicles, often surrounded by dyskeratotic cells, either isolated or in clusters (Lever & Schaumburg-Lever, 1983). Many keratinocytes and melanocytes show degenerative changes causing the migration of macrophages into the epidermis, where they phagocytose dyskeratotic keratinocytes and melanosomes (Guerrier & Wong, 1974; Caputo, Gianotti & Innocenti, 1975). These facts suggest that the three stages are related to each other and that massive infiltration of eosinophils into the epidermis may play an important role in inducing the skin lesions of IP. The generation of eosinophil chemotactic factors (CF) within the epidermis may be prerequisite for the accumulation of eosinophils.

Crusted scales are a good source of material for characterization of eosinophil chemotactic factors in the epidermis and bullous fluid (Tagami & Ofuji, 1976). There are no previous reports of the demonstration of chemotactic activity in the crusted scales of IP. To elucidate the

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mechanisms involved in the inflammatory processes in IP, we have studied the chemotactic activity of extracts of the crusted scales of IP in the vesiculobullous stage.

## METHODS

### *Patients*

Three cases of IP were studied. They were a one-week-old girl, a 3-week-old boy, and a 2-week-old girl. Shortly after birth they had had areas of erythema on the extremities. Subsequently, vesicles in linear arrangement appeared on the extremities and also, to a limited degree, on the trunk.

### *Extract of scale crusts*

Water-soluble components were prepared from the crusted scales obtained from bullous lesions. The dried scales were crushed with a Freezer/Mill (Spex Industries, Metuchen, New Jersey, U.S.A.), weighed, suspended in phosphate-buffered saline (PBS) at 20 times the original dry weight, and homogenized with a Potter-Elvehjem device, as reported by Tagami and Ofuji (1976). Extracts of callus from normal adults were used as a control.

### *Molecular-sieve chromatography*

Sephadex G-75 was used for chromatography in a column 1 × 26 cm. Scale extracts, 0·2 ml, were appled to the column and fractions of 0·5 ml were collected. The eluting buffer was PBS, and the flow rate 16 ml/h.

### *Chemotactic assay*

Eosinophil chemotactic activity of scale extracts was measured in Blind-Well Chambers (Labo Science, Tokyo, Japan), as previously described (Takematsu & Tagami, 1986). Briefly, eosinophils were obtained from the peritoneal cavity of guinea-pigs which had received repeated i.p. injections of haemocyanin. An eosinophil-rich suspension ($2 \times 10^6$ cells/ml) was incubated in chemotaxis chambers at 37°C for 3 h using 8 $\mu$m cellulose nitrate filters (Millipore Corporation, Bedford, Massachusets, U.S.A.). The cells which had migrated through the filter were counted after staining with haematoxylin and chromotrope 2R. Chemotactic activity was expressed as the number of eosinophils in 10 random high power fields.

### *Effects of boiling, enzyme digestion and ether extraction*

The fraction which showed maximal eosinophil chemotactic activity was boiled for 10 min. For enzyme digestion, the same fraction was subjected to digestion for 60 min with 5% trypsin (Sigma, St. Louis, Missouri, U.S.A.), or 5% pronase (Sigma), at 37°C in PBS, and was stopped by boiling for 10 min. For ether extraction, 0·5 ml of each fraction was adjusted to pH 3·0 with 0·5 M HCl and extracted twice into three volumes of diethyl ether (Wako Pure Chemical Industries, Tokyo, Japan). After evaporation of the ether phase, the samples were reconstituted in 0·5 ml of PBS.

### *Radioimmunoassay for LTB4*

LTB4 was assayed according to the method described by Salmon, Simmons & Palmer (1982). The radioimmunoassay kit was obtained from Amersham International U.K.

## RESULTS

### *Eosinophil chemotactic activity of scale crusts*

The eluates of the scale crusts from all three patients showed similar eosinophil chemotactic activity. A representative chemotactic profile is shown in Fig. 1. Eosinophil chemotactic activity was detected in the eluates between the vitamin B12 marker (mol. wt. 1355) and the phenol red marker (mol. wt. 364). The extracts of callus from normal adults did not show eosinophil chemotactic activity.

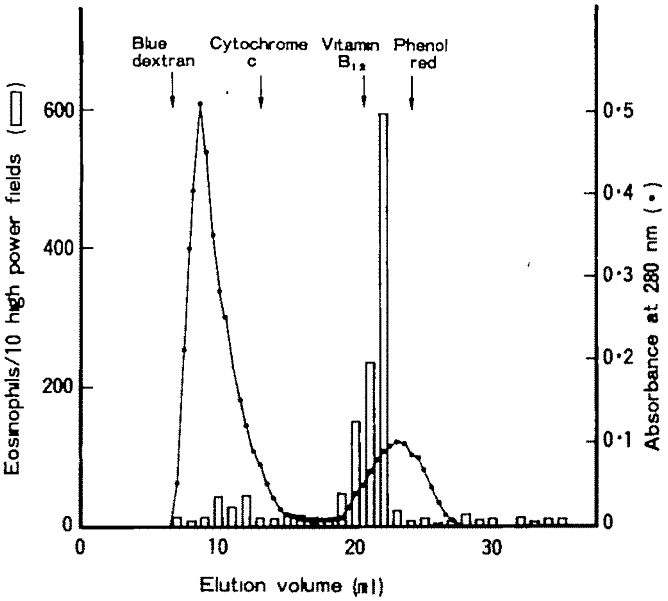

FIGURE 1. Eosinophil chemotactic activity of Sephadex G-75 fractions of extracts of crusted scales from patients with incontinentia pigmenti.

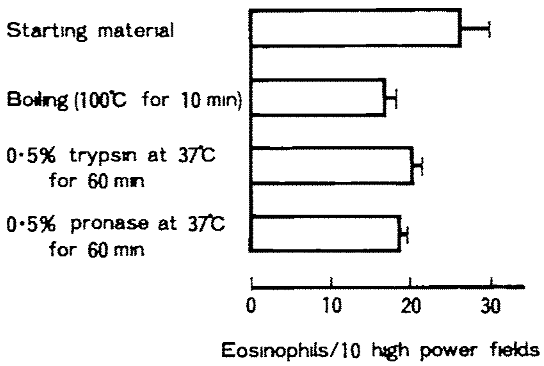

FIGURE 2. Effect of boiling and digestion with proteolytic enzymes on eosinophil chemotactic activity. Starting material was the Sephadex G-75 fraction with maximal activity. Bars represent mean ± SEM of triplicate samples.

*Characteristics of eosinophil chemotactic fraction*

The eosinophil chemotactic activity in the fraction found to have maximum activity was heat-stable and was not abolished by digestion with trypsin or pronase (Fig. 2). Ether extraction was performed on the low molecular weight fractions and recovered most of the eosinophil chemotactic activity in the eluates around the vitamin B12 marker (Fig. 3).

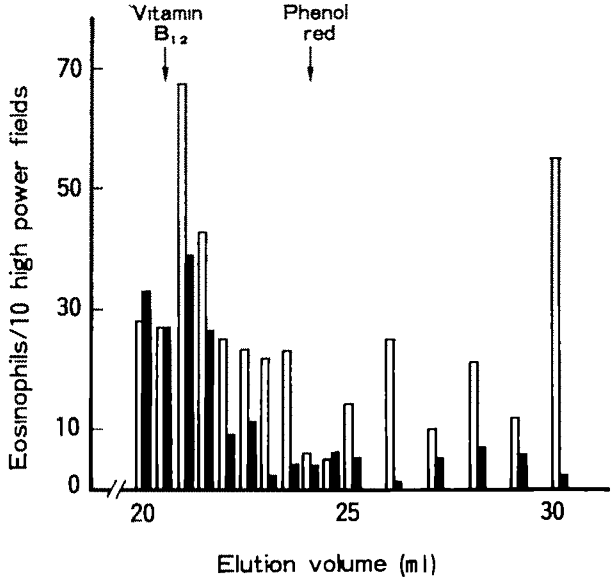

FIGURE 3. Eosinophil chemotactic activity of ether extracted ■, and control □, low molecular weight fractions of crusted scale extracts.

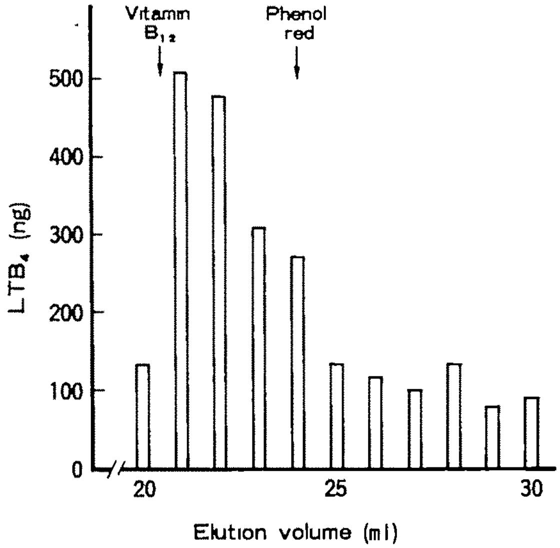

FIGURE 4. Assay of LTB4 in low molecular weight fractions of crusted scale extracts.

### *Radioimmunoassay for LTB4*

LTB4 was assayed in the low molecular weight fractions and was found to be present mainly between the vitamin B12 and the phenol red markers (Fig. 4).

## DISCUSSION

The present study indicates that the crusted scales of patients with IP in the vesiculobullous stage are capable of attracting eosinophils, and that LTB4 can be recovered from skin lesions of IP with a chromatographic elution profile corresponding to that of the eosinophil chemotactic activity. Normal stratum corneum, however, was found to have no eosinophil chemotactic activity.

Most of the eosinophil chemotactic activity eluted between the vitamin B12 and phenol red molecular markers on the Sephadex G-75 column. The low molecular weight of the eosinophil chemotactic factor suggests that chemo-attractant(s) other than lymphokine eosinophil-stimulation-promoter (ESP) (Colley, 1973) or C5a, is present.

The eosinophil chemo-attractant was found to be heat-stable and not inactivated by proteolytic enzymes. The resistance to proteolytic enzymes rules out the tetrapeptide eosinophil chemotactic factor responsible for anaphylaxis (ECF-A) (Wasserman, Goetzl & Austen, 1974). Furthermore, most of the chemotactic activity was recovered after extraction into ether at low pH. This suggests that lipid-soluble chemo-attractants may be responsible. This was supported by the demonstration of LTB4 in the eluates of the scale extracts. However, the contribution of 12-hydroxyeicosatetraenoic acid (12-HETE) or arachidonic acid to the level of LTB4 measured remains to be established (Camp *et al.*, 1985).

LTB4 has been found to be produced by keratinocytes (Rosenbach *et al.*, 1985; Camp *et al.*, (1985). On the other hand, although eosinophils from normal and hypereosinophilic donors have been observed to produce LTC4 preferentially, a substantial amount of LTB4 has been found to be generated by eosinophils (Weller *et al.*, 1983). These findings suggest that LTB4 can be generated by both keratinocytes and infiltrating eosinophils in inflammatory skin lesions. Furthermore, LTB4 is produced in skin lesions of psoriasis and other dermatoses (Rosenbach *et al.*, 1985; Camp *et al.*, 1985; Takematsu, Terui & Tagami, 1985). These findings make it doubtful that the production of LTB4 in the epidermis is a primary pathogenetic event in IP. In view of the presence of LTB4 in increased amounts in the crusted scales of IP, it may well be relevant to the maintenance of the inflammatory skin lesions of IP in the vesiculobullous stage.

Recently, Tsuda *et al.* (1985) reported the detection of two kinds of eosinophil chemotactic factors with different molecular weights (one about 1355 daltons and other much smaller) in lesional blister fluid and in a suction blister raised in clinically uninvolved skin of a patient with incontinentia pigmenti. Although they were not characterized, one of them appears to correspond to LTB4 as demonstrated in the present study. The finding that the chemotactic factors were demonstrable even in the fluid from a suction blister raised on uninvolved skin suggests that they may have been derived from dermal mast cells, as is the case with histamine (Wolf-Jurgensen, 1966).

Marked blood eosinophilia is also a prominent feature of IP. However, potent eosinophil chemotactic factors such as LTB4 or C5a have not been found to stimulate eosinophil development *in vitro* (Czarnetzki & Pawelzik, 1983). Consequently, the eosinophil chemotactic substance in the crusted scales of IP presumably plays a role only in the accumulation of circulating eosinophils within the skin lesions.

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# Use of UV–A sunbeds for cosmetic tanning

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## SUMMARY

A survey has been carried out of people using UV-A sunbeds at commercial premises in the U.K. The degree of tan achieved was found to be closely related to the subject's ability to tan in sunlight, and subjects who burnt easily in sunlight were most at risk of developing erythema after using a sunbed. Side-effects, particularly itching, were common. The prevelance of itching, nausea and skin rashes were higher in women taking oral contraceptives than in women on no medication. Although long-term quantitative estimates of the risks of UV-A sunbed use (such as skin ageing and skin cancer) are unknown, it is recommended that individuals who do not tan or tan poorly should be discouraged from using sunbeds.

The social desirability of a tanned skin is apparent, and many people associate a bronzed body with good health and a sense of well-being. This has led to the growth of suntan salons in the past decade or so, where the public are encouraged to acquire 'an attractive tan with no detrimental side-effects from the latest in ultraviolet radiation (UVR) technology'. Frequently, the manufacturers of equipment containing artificial sources of UVR may proclaim the health-giving properties of their wares and, in particular, may state that their sunlamps, sunbeds or solaria can be used for the treatment of skin diseases such as psoriasis or acne.

Concern about the use of sunbeds has been expressed by dermatologists in the U.K. (Hawk, 1983), Australia (Watson, 1982) and the U.S.A. (Bickers *et al.*, 1985), although it should be said that the long-term risks of exposure to sunbeds emitting UV-A radiation (by far the most common type in the U.K.) are, at present, only speculative.

This paper reports the results of a national survey of sunbed users in the U.K. Data are presented on the success of artificial sources of UV-A in inducing a tan, and on the frequency of short-term side-effects such as erythema, itching and nausea. In addition, the information collected concerning the pattern of use should be useful in making quantitative estimates of the possible risks of long-term effects such as skin cancer.

## METHODS

Twenty questionnaires were sent to each of 146 tanning salons in the U.K. during January 1985.

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The salon proprietors (who were all members of The Association of Sun Tanning Operators Ltd) were asked to hand one questionnaire, together with a pre-paid reply envelope, to their next 20 clients. The questionnaire comprised 24 questions and covered details including personal information (including any current medication), tanning equipment and usage, and results.

The proportions of respondents falling into particular sub-groups were compared using a chi-squared test (incorporating a continuity correction where appropriate).

## RESULTS

Between January and June 1985, a total of 1013 replies were received. It is appreciated that the design of this survey is open to criticism, as the method of distributing the questionnaires meant that a regular and satisfied sunbed user was more likely to be given a questionnaire than was an infrequent, disillusioned user. Caution should be exercised in interpreting the results since they are probably more applicable to frequent users than to users in general.

### *Subjects*

The sex and age distribution of the respondents is given in Table 1. It is clear that the practice of tanning using UV-A sunbeds in commercial premises is very much the province of young women; half of the subjects were females aged between 16 and 30 years. Sun-reactive skin types are given in Table 2. Most of the subjects said they were able to tan in sunlight.

Eighty-seven of the subjects were using a sunbed because of a skin complaint; mostly either acne or psoriasis. Table 3 indicates a fair degree of satisfaction with the results. This is difficult to equate with the conventional wisdom which suggests that exposure to UV-A alone at the typical doses achieved on a sunbed would be of little benefit to subjects with acne or eczema, and of only marginal benefit to psoriatic patients (Parrish, 1982). This apparent discrepancy may be accounted for partly by the possible bias in subject selection mentioned above.

### *Tanning equipment*

The questionnaire asked for the make of tanning equipment, the brand of ultraviolet lamp used

TABLE 1. Sex and age distribution of subjects replying to questionnaire

| | No. of subjects | |
|---|---|---|
| Age range (years) | Males | Females |
| 0–15 | 1 | 5 |
| 16–20 | 18 | 172 |
| 21–25 | 38 | 211 |
| 26–30 | 23 | 131 |
| 31–40 | 49 | 187 |
| 41–50 | 25 | 109 |
| 51–60 | 11 | 26 |
| > 60 | 3 | 4 |
| Total | 168 | 845 |

TABLE 2. Sun-reactive skin types of subjects

| Skin type | Skin reactions to sunlight | No. of subjects | % |
|---|---|---|---|
| I | Always burns easily and severely (painful burn) tans little or not at all and peels | 154 | 15 2 |
| II | Usually burns easily and severely (painful burn) tans minimally or lightly, also peels | 282 | 27 8 |
| III | Burns moderately and tans moderately | 415 | 41 0 |
| IV | Burns minimally, tans easily | 162 | 16 0 |
| Total | | 1013 | 100 |

TABLE 3 Numbers of subjects satisfied with treatment of skin complaint

| Skin Disease | Degree of satisfaction Complete | Partial | None | Total |
|---|---|---|---|---|
| Psoriasis | 17 | 5 | 0 | 22 |
| Acne | 27 | 13 | 0 | 40 |
| Eczema | 10 | 4 | 0 | 14 |
| Other | 7 | 4 | 0 | 11 |
| Total | 61 | 26 | 0 | 87 |

in the equipment and type of ultraviolet rays emitted (UV-A or UV-B). However, not surprisingly, a large proportion of subjects failed to answer these questions. Using the answers that were supplied, and inference from the exposure time per session in the remaining subjects, it was apparent that all the equipment used emitted predominantly UV-A radiation (315–400 nm) rather than UV-B radiation (280–315 nm) as emitted by the older type of 'prescription' mercury arc sunlamp. Most of the sunbeds used incorporated UV-A fluorescent lamps, although about 10% of the sunbeds contained optically-filtered high pressure metal halide lamps (Diffey, 1982).

### *Ultraviolet exposure*

A large proportion of subjects (61%) used a sunbed for a half hour session, although exposure times ranged from 10 to 60 min. This probably reflects economic rather than photobiological considerations since it is unlikely that the operators attempted to give their clients a fixed UV-A dose per session, and probably in the majority of cases no allowance was made for a reduction in UV-A irradiance due to lamp ageing. Other factors which govern the UV-A irradiance at the users' skin are the design of the sunbed, the number of lamps used, and their electrical power and spectral emission.

The number of sunbed sessions per subject ranged from one to approximately 2000 (Table 4). The distribution of numbers of sessions showed a pronounced positive skew with a median of 20 sessions.

TABLE 4. Approximate number of sessions for which subjects had used a sunbed

| No. of sessions | No. of subjects | % |
|---|---|---|
| ≤3 | 55 | 6·3 |
| 4–10 | 240 | 27·3 |
| 11–30 | 303 | 34·5 |
| 31–100 | 203 | 23·1 |
| 101–300 | 61 | 6·9 |
| 301–1000 | 16 | 1·8 |
| 1001–3000 | 1 | 0·1 |
| Total | 879 | 100 |

Seventy-eight percent of subjects reported that they always wore goggles when using a sunbed; 14% sometimes wore them and 8% never wore them. The fact that goggles are not always worn by everyone is probably due to reluctance for cosmetic reasons (the so-called 'panda look') coupled with ignorance on the part of the users concerning the long-term risks of UV-A exposure to the lens of the eye. Epidemiological studies suggest that human cataracts may be related to sun exposure, although a clear demonstration of the role of UV-A exposure in the aetiology of human cataracts is lacking (Parrish *et al.*, 1978). However, the topology of the face is such that the eye is normally well protected from direct sunlight by the eyebrows, and measurements of solar UV levels on a model head (Diffey, Tate & Davis, 1979) indicate that the UV-A irradiance on the surface of the eye when looking directly at a UV-A sunbed could be as much as two orders of magnitude higher than when outdoors in sunlight.

*Skin changes*

Table 5 shows that virtually all subjects (98%) reported a slight or deep tan after using a sunbed. This is considerably higher than in the controlled study carried out on volunteer hospital staff (Devgun, Johnson & Paterson, 1982) in which some tanning had occurred in 20 out of 33 subjects (60%) following 10 exposures of the whole body over a 2-week period. One reason for this may have been that a biased population replied to the questionnaire; those people who had tried a sunbed but found it ineffective would presumably not be attending the tanning salon and

TABLE 5. Tan achieved with sunbed related to tanning in sunlight

| Ability to tan in sunlight | Degree of tan with sunbed | | |
|---|---|---|---|
| | Deep | Slight | None |
| Easily | 295 | 317 | 4 |
| Slightly | 84 | 265 | 12 |
| Never | 3 | 13 | 6 |

$\chi^2$ test: $P < 0{\cdot}001$.

would therefore not complete a questionnaire. What is apparent from Table 5 is that of the 22 subjects who never tanned in sunlight, six (27%) were unable to tan on the sunbed. On the other hand, only 4 (0·6%) of the 616 subjects who tanned easily in sunlight achieved no tan on the sunbed. The proportion of subjects who tanned slightly in sunlight but achieved no tan on the sunbed was 3%.

There was also a definite relationship between development of erythema after sunbed exposure and the subject's experience of burning in sunlight (Table 6). Sixty-five (29%) of subjects who burnt easily in sunlight developed erythema after using a sunbed, compared with 6% of those subjects who never burnt in sunlight.

*Possible psychological benefits*

A high proportion (83%) reported that they had felt more relaxed since using a sunbed (Table 7). Whether there is a biological effect of light on the central nervous system remains conjectural, although artificial light has been used successfully in the treatment of psychiatric illness (Lewy *et al.*, 1982). Certainly, many people would admit that their morale is higher on blue skied sunny days than on overcast rainy days. However, the apparent psychological

TABLE 6. Number of subjects experiencing erythema due to sunbed exposure

| Do you sunburn in sunlight? | Has your skin ever gone red following sunbed exposure? | | |
|---|---|---|---|
| | Yes | No | Don't know |
| Easily | 65 | 158 | 2 |
| Slightly | 114 | 480 | 6 |
| Never | 11 | 169 | 2 |

$\chi^2$ test: $P < 0{\cdot}001$.

TABLE 7. Psychological response to sunbed exposure

| Degree of tan achieved with sunbed | Do you feel more relaxed, more self confident or happier since using a sunbed? | | |
|---|---|---|---|
| | Yes | No | Don't know |
| Deep | 338 | 24 | 20 |
| Slight | 478 | 60 | 53 |
| None | 10 | 7 | 3 |

$\chi^2$ test: $P < 0{\cdot}001$.

benefits were closely related to the degree of tan that was achieved on the sunbed (Table 7) and it may be that those subjects who obtained the desired tan were naturally satisfied with the outcome.

### *Side-effects*

The incidence of acute side-effects was substantial: 28% of subjects complained of itching either during or immediately after exposure; and about 8% of subjects had developed a skin rash or had felt nauseous at some time during or immediately after exposure. There was no evidence that any of these three symptoms was related to the sex of the subject. Neither was there reason to suppose that the cosmetic preparations worn by the subjects increased their risk of side-effects, despite the fact that 131 subjects stated that they used a preparation on their skin before using a sunbed. Occasionally this was eye shadow or lipstick, but in the majority of cases the preparations used were skin moisturisers, e.g. Uvamed® 'Bruiningscreme' intended '... for sunbench, solarium and fast tanning systems'. There is, of course, a risk of developing berloque dermatitis or 'suntan salon photodermatitis' (Larsen, 1985) if perfumes containing oil of bergamot are used on skin which is subsequently exposed to UV-A radiation.

One hundred and ninety five subjects (19%) were taking prescribed medication whilst using a sunbed. Apart from the 136 females taking oral contraceptives, 12 subjects or fewer were taking each of any other group of drugs. These numbers were too small to permit conclusions to be drawn regarding drug-induced photosensitivity. In addition, 35 subjects were taking non-prescription vitamins or tonics.

It has been suspected for some time that oral contraceptives may cause photosensitivity in some individuals (Elgart & Higdon, 1971; Horkay *et al.*, 1975) where it is thought that the oestrogen component of the medication may be implicated. The results of the present study strongly suggest that females who take oral contraceptives (OC) and use a sunbed are more likely to experience side-effects, particularly itching, than females who are taking no medication (Table 8). Both groups of females (no drugs, and OC users) were well matched in terms of their experience of tolerating sunlight (Table 9). Table 10 shows that the incidence of nausea was significantly lower in subjects who always wore goggles than in those who sometimes or never wore them ($P<0{\cdot}02$). UV-A absorbed by the lens of the eye results in the emission of fluorescent blue light which can lead to visual disturbance and possibly cause nausea.

TABLE 8. The incidence of itching, nausea and skin rashes in females aged between 16 and 45 who were taking oral contraceptives compared with those taking no systemic medication

| | Number of females experiencing | | | | | |
|---|---|---|---|---|---|---|
| | Itch* | | Nausea† | | Rash† | |
| Drug | Yes | No | Yes | No | Yes | No |
| None | 150 | 411 | 55 | 505 | 40 | 523 |
| Oral contraceptive | 53 | 76 | 22 | 105 | 18 | 113 |

$\chi^2$ test: *$P<0{\cdot}005$; †$P<0{\cdot}025$.

TABLE 9. Sun-reactive skin type of females aged between 16 and 45 who were taking oral contraceptives and those taking no systemic medication

| | Sun-reactive skin type | | | |
|---|---|---|---|---|
| Drug | I | II | III | IV |
| None | 72 | 164 | 244 | 85 |
| Oral contraceptive | 20 | 35 | 60 | 17 |

$\chi^2$ test: not significant.

TABLE 10. Numbers of subjects who wore eye protection and the numbers experiencing nausea

| | Nausea ever experienced | |
|---|---|---|
| Goggles worn | Yes | No |
| Always | 66 | 721 |
| Sometimes/ never | 30 | 178 |

$\chi^2$ test: $P < 0.02$.

## DISCUSSION

Although almost one third of respondents indicated that they had experienced one or more side-effects, notably itching, at some time while using a sunbed, in most cases these effects were transient and mild. In some cases, the effects may not have been photobiological in origin, but could be related to the heat and humidity associated with sunbed use. Nevertheless, those at most risk of developing skin irritation, erythema or eczema, often followed by unsightly pigmentation include the following:

(i) people who sunburn rather than tan in sunlight (skin types I and II);
(ii) subjects with an idiopathic photodermatosis such as polymorphic light eruption or solar urticaria;
(iii) subjects with a pre-existing condition (such as lupus erythematosus or rosacea) which can be aggravated by exposure to UVR;
(iv) subjects who are applying potentially photosensitizing substances to their skin, notably psoralen-containing perfumes, or who are receiving therapeutic medication known to be

associated with adverse cutaneous drug photosensitivity. The most common systemic photosensitizers include drugs prescribed for hypertension, bacterial infections, and cardiac, renal, joint and mental disorders (Hawk, 1984).

What are the long-term risks of UV-A sunbed exposure? There is evidence, from studies in hairless mice, that UV-A can induce epidermal tumours (van Weelden, de Gruijl & van der Leun, 1983) and profound photodamage within the dermis (Kligman, Akin & Kligman, 1985). Furthermore, immunological changes have been observed in the skin of subjects who used a sunbed for 12 half-hour sessions during a 2-week period (Hersey *et al.*, 1983), and it was suggested that the changes noted could be expected to impair host defences against the induction of skin tumours.

The UV-A irradiance at the skin of a subject lying on a sunbed incorporating UV-A fluorescent lamps is likely to be in the range 4–12 mW/cm², whereas with a sunbed incorporating high power metal halide lamps, UV-A irradiances of up to 40 mW/cm² at the subject's skin can be expected (Diffey, unpublished observations). Measurements of the UV-A present in daylight indicate that around midday during the summertime in the U.K., maximal levels of about 4–5 mW/cm² are obtained (Moseley, Davison & MacKie, 1983). These values, coupled with measurements of personal exposure to sunlight (Diffey & Larkö, 1984), indicate that the UV-A irradiance on the hands and face of a subject walking about outdoors in the summertime is approximately 1 mW/cm². So a subject using a sunbed containing the common UV-A fluorescent tubes (e.g., Philips TL/09, Wolff relarium, Wotan L100/79, Osram Liteguard) is exposed to approximately twice the intensity of UV-A that he would be if sunbathing on a beach around midday in midsummer. However, summer sunlight is much richer in UV-B radiation (Diffey & Larkö, 1984) than are UV-A fluorescent tubes (Diffey & McKinlay, 1983) and it is the UV-B waveband in sunlight that is known to be primarily responsible for the induction of non-melanoma skin cancers (Urbach, Epstein & Forbes, 1974). In principle, by combining the spectrum of either summer sunlight or the emission from a UV-A sunbed with the relative effectiveness of different wavelengths (action spectrum) in inducing skin cancer, it is possible to calculate the relative carcinogenic potential of these two sources. The action spectrum for skin carcinogenesis in humans is clearly not available, but in keeping with most work on photocarcinogenesis, it is assumed that the action spectrum for UV erythema in human skin is an adequate approximation for the purpose of the present comparison (van der Leun, 1984). The results of these calculations indicate that the carcinogenic risk of a 30 min session on a sunbed incorporating UV-A fluorescent lamps with a UV-A irradiance at the skin surface of 8 mw/cm² is equivalent to sunbathing for about 10 min on an English beach on a clear day around midday in midsummer. During this period a Caucasian with skin type II receives about one half of a minimal erythema dose (MED); one MED being that dose of UVR required to produce a just perceptible reddening of unacclimatized skin at 8–24 h after exposure.

Recently, a new type of UV-A fluorescent lamp (Philips TL/10R, also known as R-UVA) has been developed specifically for cosmetic tanning purposes. This lamp has a UV-A irradiance which is about twice that of the well-established lamps used in sunbeds (e.g. Philips TL/09) but with the short wavelength end of the fluorescence spectrum extending only to 345 nm rather than 310 nm as with the currently widely-used UV-A lamps. These lamps are presumed to be safer than the TL/09 type of UV-A lamp because the UV-B component of the TL/10R lamp is less than 0·1% of the UV-A component (Diffey, unpublished measurements), whereas with the TL/09 and similar types of lamp, the UV-B component is about 1% of the UV-A component (Diffey & McKinlay, 1983). Yet, in order to achieve the same 'tanning dose' as would be obtained by lying on a conventional UV-A sunbed for 30 min (TL/09 type of lamp with a UV-A irradiance at the skin surface of 8 mW/cm$^2$), the exposure time on a sunbed incorporating TL/10R lamps would need to be 40 min, even though the UV-A irradiance is assumed to be 20 mW/cm$^2$. The reason for this is that the melanogenic effectiveness of UVR increases with decreasing wavelength in the region 400–300 nm (Parrish, Jaenicke & Anderson, 1982). More importantly, the erythemally-effective (and presumably carcinogenic) UVR dose is the same for both these exposure conditions, since the erythema action spectrum is virtually identical to the melanogenesis action spectrum in the 300–400 nm region (Parrish *et al.*, 1982). It should be pointed out that the subjects in whom these action spectra were obtained were genetically poor tanners. It remains to be seen whether similar action spectra are obtained in subjects who tan well.

The long-term effects on the skin, of UV-A sunbed use in people who tan easily (skin types III and IV), remain uncertain. However, individuals who do not tan or tan poorly (skin types I and II) should be discouraged from exposing their skins to any source of ultraviolet radiation. Not only is there evidence that this group is likely to be disappointed with the results of attempts at cosmetic tanning (Table 5), but also they may well end up with red, rather than the desired tanned, skin (Table 6). Furthermore, it is this population that is genetically most susceptible to the development of degenerative and neoplastic changes in the skin as a result of chronic sun exposure (Urbach *et al.*, 1974), and there is good reason to believe that the additional exposure occurring from UV-A sunbeds will augment the damaging effects of sun exposure. Finally, malignant melanoma has developed in subjects who have used sunlamps or sunbeds (Retsas, 1983). It is uncertain how much, if at all, their sunbed exposure contributed to the development of their tumours, although there is some epidemiological evidence to suggest that short, intense episodes of sunlight exposure resulting in burning may be one of the aetiological factors involved in the development of melanoma (MacKie & Aitchison, 1982).

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***Pharmacology and Treatment***

# Treatment of polymorphous light eruption with nicotinamide: a pilot study

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## SUMMARY

In a pilot study, 42 patients suffering from polymorphous light eruption (PLE) were treated with oral nicotinamide, 3 g daily, for 2 weeks. Twenty-five patients remained free from lesions despite extensive sun exposure. We suggest that an abnormality in tryptophan metabolism is important in the aetiology of PLE, and that nicotinamide administration partially corrects this.

The aetiology of polymorphous light eruption (PLE) is still unknown and therapy remains empirical.

Although PUVA therapy and systemic corticosteroids seem to provide the best treatment results, systemic therapy with beta carotene and antimalarial drugs has been reported to be of value (Jansen, 1974; Epstein, 1960). It has been suggested that errors in tryptophan metabolism may play a role in photodermatoses (Sohier *et al.*, 1979; Binazzi & Calandra, 1971. Kobori *et al.*, 1974). Metabolism of tryptophan is an important source of the vitamin nicotinamide, the other source being dietary. On this basis, we conducted a pilot study to evaluate the efficacy of oral nicotinamide in patients suffering from PLE. Data available on the possible side-effects of nicotinamide administration suggest that even in high doses (up to 5 g daily) it is very safe, the only reported side-effect being fatigue. However, it has to be avoided in diabetic subjects.

## METHODS

### *Patients*

Forty-two patients (39 female, 3 male; aged 16–56 years) entered the study. All had a history of PLE for more than two years, a PLE being defined as a recurrent pruritic eruption provoked by sunlight and confined to exposed skin, unrelated to drug use, the lesions lasting from 24 h to 10 days and disappearing without leaving scars.

The predilection sites were the V-shaped area on the upper chest (35 patients), the extensor surface of the upper arms (33 patients), the forearms (28 patients), the neck (23 patients), the face (19 patients) and the lower legs (13 patients). Tests for anti-nuclear factors and Ro and La

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titres were negative, and blood and urine testing showed no evidence of porphyrin disorders.

Patients had not benefited from topical application of sun-screening agents with sun-protection factors ranging from 6 to 17.

*Treatment*

Nicotinamide was given at a dosage of 1 g orally three times a day for 2 weeks, and treatment commenced 2 days before any sun exposure. If during the first week no lesions occurred, the total daily dose was decreased to 2 g, and the dose was increased to 3 g/day again in case of recurrence. We also instructed the patients to stop treatment after 3 days of sun exposure if it proved ineffective. Patients kept a daily record of the appearance of skin lesions and any side effects. Prior to starting therapy, a full blood count, and tests for transaminases, glucose, blood urea-nitrogen and serum creatinine were performed.

## RESULTS

The results of this treatment are presented in Table 1. Twenty-five patients (60%) reported excellent response to nicotinamide; despite daily sun exposure no itching or lesions developed.

TABLE 1. Responses of 42 patients with PLE to treatment with nicotinamide

| | No. of patients | | | |
|---|---|---|---|---|
| Type of lesions | Excellent | Marked | None | Total |
| Papular | 20 | 2 | 8 | 30 |
| Papulovesicular | 5 | 3 | 2 | 10 |
| Urticarial | 0 | 1 | 1 | 2 |
| Total | 25 | 6 | 11 | 42 |

In 14 of these patients, 2 g daily was also effective, while 11 patients developed mild recurrence at the decreased dose. Complete remission was obtained again with 3 g daily. Six patients (14%) reported a marked improvement compared with former PLE-attacks; the itching was diminished and the intensity of the lesions was less. Eleven patients (26%) did not benefit from nicotinamide treatment.

The nicotinamide was tolerated very well. No side effects were reported, except mild fatigue in five patients. Serum transaminases, glucose, blood urea-nitrogen and serum creatinine were within the normal range after treatment.

## DISCUSSION

The present study indicates that oral nicotinamide was effective in preventing or alleviating PLE in 60% of our patients at a dosage of 2–3 g daily.

The effect seemed to be dose-dependent, because 11 patients developed a recurrence when they decreased the daily dose from 3 g to 2 g. Studies on the pharmacokinetics of nicotinamide

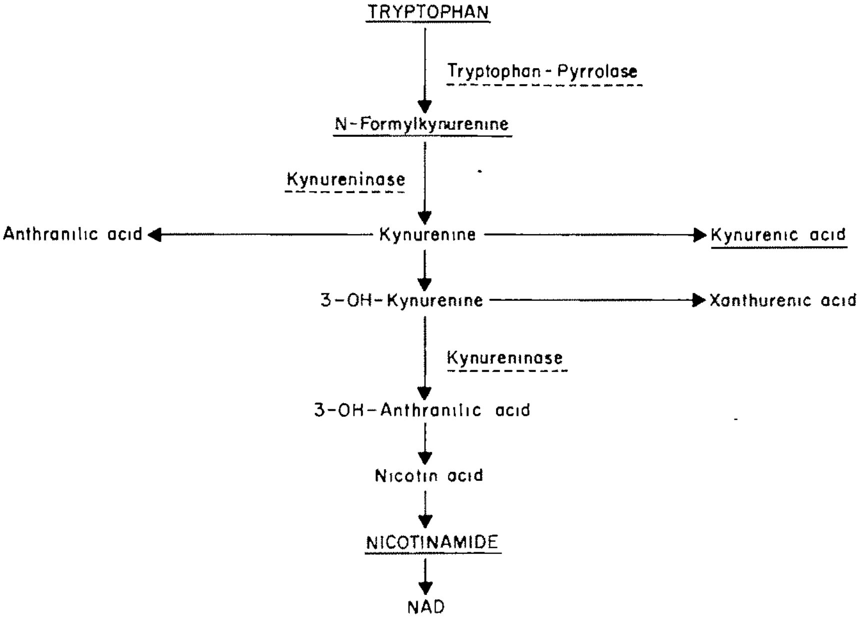

FIGURE 1. Catabolism of tryptophan to nicotinamide.

are needed to determine the effective therapeutic serum levels and the appropriate duration of nicotinamide treatment prior to sun exposure.

A purely clinical evaluation of the effect of a solar-protective agent is rather difficult, but diary recording of itching and skin eruptions is a practical and probably realistic way of assessing patients' responses (Corbett, 1982). Every study on PLE must take into account the so-called 'hardening' effect, an increased resistance to sunlight as the summer progresses. This was reported by 10 patients in our study. However, therapy was started at the beginning of the summer, so we may exclude any influence of this 'hardening' effect on our results.

Other factors complicating the assessment of our results are the lack of standardization of light exposure, because the solar intensity and the time the patients spent out of doors varied considerably, and a possible placebo effect of nicotinamide. A double-blind controlled study with artificial light testing is now needed.

We suggest that PLE may be caused by an error in tryptophan metabolism (Sohier *et al.*, 1979; Binazzi & Calandra, 1971; Kobori *et al.*, 1974) resulting in accumulation of kynurenic acid (Fig. 1). This may cause a phototoxic reaction in irradiated skin areas (Swanbeck & Wennersten, 1973; Wennersten & Brunk, 1977). The presence of a photosensitizer in the circulation might lead to a direct interaction of light with the vascular endothelium with destruction of these cells (Gschnait, Wolff & Konrad, 1975; Konrad *et al.*, 1975). Nicotinamide is capable of blocking the formation of kynurenic acid, the putative photosensitizer in PLE (Cho-Chung & Pitot, 1968), and we consider that its use in the treatment of this disorder merits further study.

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# The surgical treatment of upper limb hyperhidrosis

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## SUMMARY

Primary hyperhidrosis is a common and poorly understood condition. Surgical thoracic sympathectomy, either by the cervical or transaxillary route, entails major surgery, so there is a tendency to offer this only to those most severely affected. Endoscopic thoracic sympathectomy is a simple, safe and effective procedure. The technique and results are described and it is recommended as the appropriate procedure for treating upper limb hyperhidrosis, including localized axillary sweating.

Primary hyperhidrosis is a common and distressing condition of unknown aetiology defined by Kux (1978) as a 'pathological condition in which there is excessive sweating beyond that needed to cool the body'. Sweating starts in adolescence and has been present usually for many years before patients seek medical help.

Hyperhidrosis is characterized by a massive outpouring of secretions from the eccrine sweat glands. Though these are widely distributed throughout the body, patients primarily complain of the sweating affecting the hands, feet and axillae. When offered a choice of the area to be treated, the majority of patients opt for treatment of the upper limbs (Greenhalgh, Rosengarten & Martin, 1971).

Localized axillary sweating may be controlled with topical aluminium chloride (Scholes *et al.*, 1978) and palmar sweating may be controlled locally with iontophoresis using tap water or anticholinergic drugs (Abell & Morgan, 1974). Permanent cessation of hyperhidrosis may follow these treatments. Local surgery to excise the eccrine glands is also effective but may cause severe scarring (Ellis, 1977). However, in upper limb hyperhidrosis the hands alone are affected in 20% of cases, the hands and axillae in 43% and the axillae alone in 37%. Therefore, for the majority of patients, treatment must be 'systemic' to control the entire upper limb.

Anticholinergic drugs are unsatisfactory as they are of little use for axillary sweating and have unpleasant side-effects. Sympathectomy is an excellent treatment but at present is reserved for the severest cases as it is considered a major undertaking. There are two common approaches to upper limb sympathectomy, 'cervical' and transaxillary. Greenhalgh *et al.* (1971) described a

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series of 28 patients treated with bilateral cervical sympathectomy. The incidence of permanent Horner's syndrome was 4%, and 37% of patients continued to have excessive axillary sweating. This approach endangers the stellate ganglion and makes it difficult to reach the fourth thoracic ganglion which must be destroyed to control the axilla. Transaxillary sympathectomy provides a simpler anatomic approach to the chain and the fourth ganglion is easily accessible. Ellis (1979) described a series of 62 patients thus treated: 61 of them obtained complete relief of their symptoms and there was no case of Horner's syndrome.

Transaxillary sympathectomy, although the standard operative treatment for upper limb hyperhidrosis, has disadvantages. It may be safest to operate on one side at a time so patients require two hospital admissions. Protagonists of transaxillary sympathectomy reserve the operation for patients with both axillary and hand sweating, employing more local measures for axillary sweating alone (Ellis, 1977).

A simple, safe and effective treatment applicable to patients with both axillary and palmar sweating is needed. Kux (1978) reported on 124 thoracic endoscopic sympathectomies for hyperhidrosis in 63 patients, of whom 55 were entirely satisfied. Post-operative discomfort and scarring were minimal and the hospital stay was short. The technique has not gained widespread use or acceptance and there are only three recent reports in the literature. Weale (1980) described its use for the first time in the U.K. on a small number of patients, some of whom had hyperhidrosis. Malone, Duignan & Hederman (1982) performed 34 sympathectomies in 17 patients with hyperhidrosis with complete success and no complications. The hospital stay was three days and patients returned to work within a week. Horgan *et al.* (1984) reported similar impressive results in 70 patients with hyperhidrosis treated by this method.

The purpose of this paper is to describe our initial experience in five patients with hyperhidrosis.

## SURGICAL TECHNIQUE

A pre-operative chest X-ray is essential to exclude pulmonary pathology which might have caused pleural adhesions as these are a relative contra-indication to the procedure. A standard Olympus double-puncture laparoscope was used.

The patients are anaesthetized using a double-lumen endotracheal tube and positioned supine with both arms abducted at right angles. A 45-degree head up tilt allows the lung to fall away from the operative field. Through a small incision in the third intercostal space in the anterior axillary line the Verrez needle is introduced after the appropriate side of the endotracheal tube has been clamped and disconnected. A pneumothorax is produced using 1·5 litres of $CO_2$ and the laparoscope is inserted. The sympathetic chain is visualized running vertically over the necks of the ribs, from the second to the fifth thoracic ganglion. It is not possible to see the stellate ganglion.

The diathermy forceps are inserted through a separate stab incision in the fourth intercostal space and the chain is coagulated and destroyed from the second to the fifth ganglion. Following satisfactory coagulation, the pneumothorax is released and the lung is inflated under direct vision. The same procedure is repeated on the other side. The skin incisions are closed with a single suture. A post-operative chest X-ray is performed in the recovery room.

## PATIENTS AND RESULTS

### *Patient 1*

A 24-year-old female law student who had suffered from hyperhidrosis of her axillae, hands and feet since adolescence opted to have upper limb sweating treated. A unilateral endoscopic sympathectomy was performed without difficulty and with complete success. Post-operatively she suffered a mild transient Horner's syndrome which had cleared by the time of discharge 48 h later. She suffered little post-operative discomfort and returned to her studies in 5 days. Endoscopic sympathectomy was repeated on the opposite side 6 weeks later, again without difficulty, but once again she suffered a transient Horner's syndrome. The patient is delighted with the result and 9 months later her hands and axillae are still dry.

*Patient 2*

A 23-year-old male muscian had had axillary and palmar sweating for years which interfered with his job considerably. He had unilateral endoscopic sympathectomies performed 6 weeks apart without difficulty or complications and with complete success. Hospital stay was 3 days for each procedure and the patient returned to work in a week; 8 months later he remains free from symptoms.

*Patient 3*

A 24-year-old man had suffered axillary and palmar sweating for as long as he could remember. Once again, bilateral sympathectomy was performed on separate occasions 4 weeks apart. There were no difficulties or complications and the patient experienced little post-operative pain. His hospital stay was 3 days on each occasion and he also returned to work within a week. He remains free of symptoms 8 months later.

*Patient 4*

A 36-year-old male catering manager had had axillary and palmar sweating since adolescence. He had a simultaneous bilateral endoscopic sympathectomy performed and 2 months later his hands and axillae are dry. On one side minimal pleural adhesions were encountered but these were divided with ease. Hospital stay was 3 days and the patient was back at work in a week.

*Patient 5*

A 44-year-old man had had axillary hyperhidrosis for years. Simultaneous bilateral sympathectomy was attempted. On one side pleural adhesions were encountered but it was possible to divide these and proceed with the sympathectomy. On the opposite side dense pleural adhesions were present and the procedure was abandoned. A small puncture wound was made in the lung and a chest drain was inserted for 24 h, but there were no long-term problems and the patient was discharged 3 days post-operatively. The sympathectomy performed was successful.

As this series describes a learning experience, the time taken to perform endoscopic sympathectomy was longer than previously recorded, but we would agree with Malone *et al.* (1982) and Horgan *et al.* (1984) that with practice it should be possible to complete a bilateral procedure within an hour.

## DISCUSSION

Our initial experience with endoscopic thoracic sympathectomy supports the view that it is a simple, safe and effective procedure. We have followed patients for up to 9 months and the effects of sympathectomy persist, suggesting that the long-term results will be similar to those of open operation. This agrees with previous studies (Kux, 1978; Malone *et al.*, 1982; Horgan *et al.*, 1984). One patient developed a bilateral temporary Horner's syndrome, which was reported also by Weale (1980). There was no case of post-operative pneumothorax and patients experienced little post-operative discomfort. We have not seen the compensatory sweating on the trunk described by others (Kux, 1978; Weale, 1980).

The operation is easily learned. Only one of us had previous experience, but following initial demonstration it was easy to perform safely and quickly. We encountered only one difficulty: finding dense pleural adhesions in a patient with a normal chest X-ray. These led to a small puncture of the lung and the patient required a chest drain for 48 h but suffered no long-term problem. Finding dense adhesions in the presence of a normal chest X-ray is unusual and this rare possibility should not act as a deterrent (Ellis, 1979). Minor and moderate pleural adhesions are seen more commonly but it is possible to dissect these endoscopically and proceed. The instrument used is the standard laparoscope as employed by the gynaecologists and so is readily available in most hospitals.

From our initial experience, we suggest that endoscopic thoracic sympathectomy should be considered for many cases of upper limb hyperhidrosis including those with sweating confined to the axillae. We hope the procedure will become widely accepted and used.

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***Case Reports***

# Concurrent multiple morphea and neonatal lupus erythematosus in an infant boy born to a mother with SLE

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## SUMMARY

An infant boy born to a mother with systemic lupus erythematosus developed multiple morphea and annular erythematous lesions. The annular lesions appeared on his face, back, chest and extremities one month after birth and faded within 7 months. The sclerotic lesions progressed until the age of 6 months and remain on his back, chest, right cheek, the nape of the neck and left shoulder at the age of 3 years.

Histological findings in the sclerotic lesions were consistent with scleroderma. Serological tests, performed at 14 months of age, were within normal limits. The mother developed acute SLE and died of cardio-renal failure 8 months after the delivery.

It is highly probable that maternal factors transferred through the placenta caused the sclerotic lesions in the infant.

Since first described by McCuistion and Schoch (1954), over 100 cases of neonatal lupus erythematosus have been reported (Lumpkin *et al.*, 1985). Neonatal LE is characterized by annular erythemas which fade within a few months of their first appearance (Vorderheid *et al.*, 1976).

It has been reported that the complications of neonatal LE are congenital atrioventricular block, haematological abnormalities and hepatosplenomegaly (Provost, 1983; Draznin *et al.*, 1979). Some patients born with neonatal LE have developed systemic LE later (Lumpkin *et al.*, 1985). However, there are no reported cases of multiple morphea and neonatal LE occurring concurrently.

We describe here a patient with multiple morphea and neonatal lupus erythematosus born to a mother with SLE.

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## CASE REPORT

A 14-month-old boy was transferred from Fuchu Hospital to our clinic in March 1983, suffering from atrophic and sclerotic lesions on his face, back and chest.

He was born at term, after an uneventful pregnancy, to a mother who had systemic lupus erythematosus. At birth his skin was normal. At the age of one month, annular erythemas 1–2 cm in diameter appeared on his face, back, chest, abdomen and legs. Most of these annular erythemas faded within the first 7 months of life. However, some annular lesions had central atrophy and enlarged gradually up to palmar size. The halo of erythema faded within the first 1–2 months, leaving large atrophic lesions which gave rise to areas of sclerosis by the age of 2 months (Fig. 1).

Examination of the skin at the age of 14 months revealed well circumscribed sclerotic plaques with a partially depigmented or hyperpigmented, slightly shiny centre, on the back, right cheek, the nape of the neck, left shoulder and left side of the chest. Physical examination showed no other abnormalities. All laboratory findings, including a serological study carried out at the age of 14 months, were within normal limits. Anti SSA and SSB antibodies were negative. An ECG was normal.

The first biopsy of the atrophic lesion on the back, taken at 2 months of age, revealed focal areas of mild liquefaction degeneration of the basal layer and a mild lymphocytic infiltrate in the perivascular space and between the slightly thickened collagen bundles of the dermis. Newly formed collagen fibres replaced subcutaneous tissue (Fig. 2).

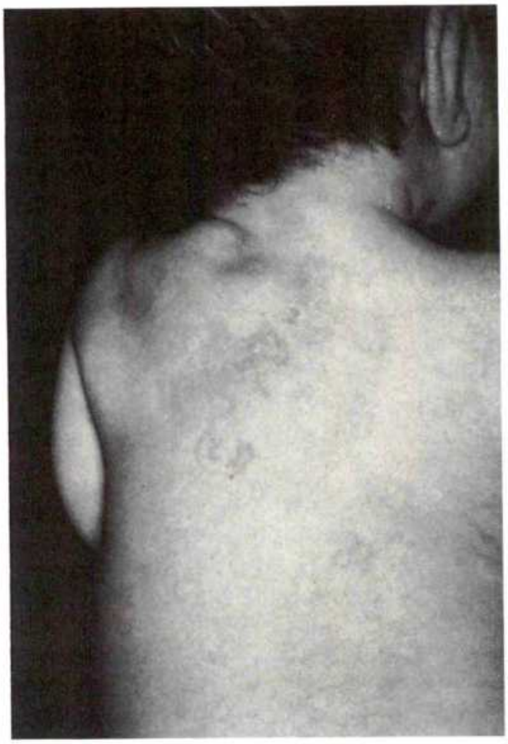

FIGURE 1. Annular lesions and sclerotic plaque on the back at 2 months of age.

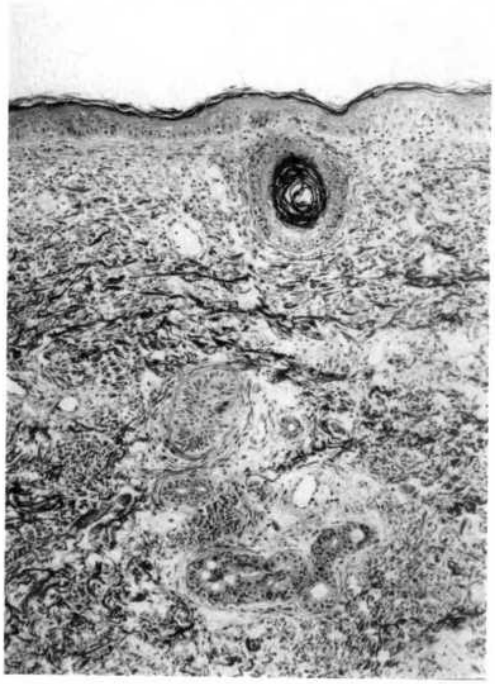

FIGURE 2. First biopsy (at 2 months of age). Focal liquefaction degeneration of the basement membrane of the epidermis and a mild perivascular cell infiltrate are seen in the specimen. Collagen fibres of the dermis are slightly thickened.

In the second biopsy of the same lesion, taken at 14 months of age, the epidermis was normal, and the inflammatory infiltrate had almost completely disappeared. The eccrine glands appeared atrophic, and fat was replaced by collagen, both in the subcutaneous tissue and the tissue surrounding the eccrine glands (Fig. 3). The third biopsy, taken at 3 years of age, showed

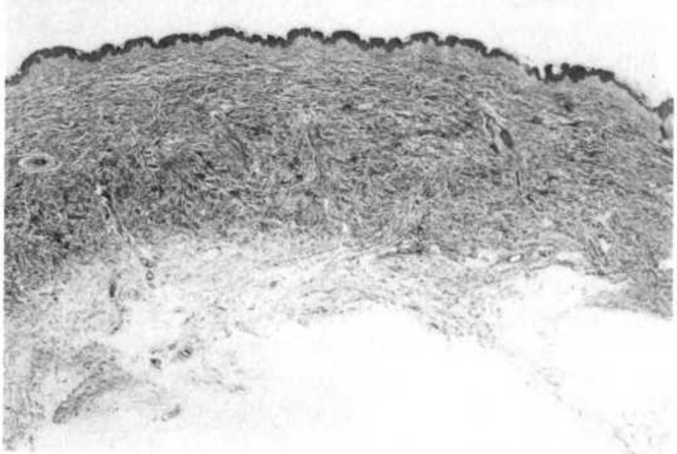

FIGURE 3. Second biopsy (at 14 months of age). The subcutaneous fat has been replaced by fine collagen. The eccrine glands are atrophic and surrounded by collagen fibres.

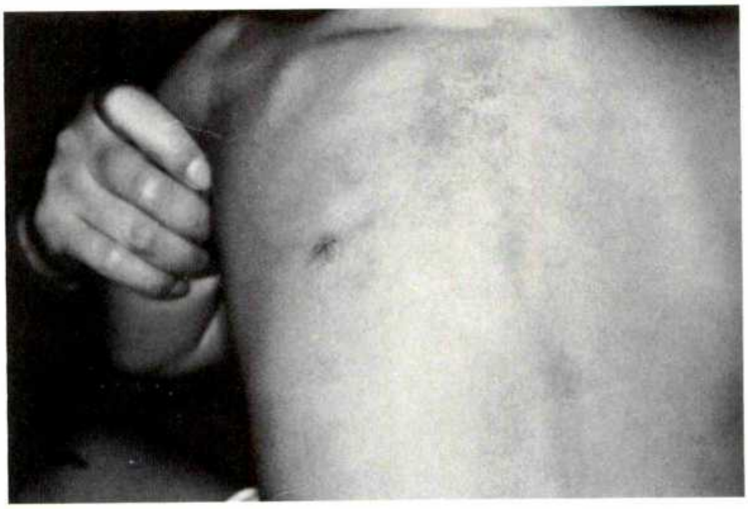

FIGURE 4. Sclerotic plaque on the back at 3 years of age.

the same features as the second. Direct immunofluorescence of the second and third biopsy specimens showed no deposits of IgG, IgM, IgA, IgE, C3 or fibrinogen.

The patient, now 3 years old, still has the sclerotic lesions which have not enlarged since the first examination at 14 months. The sclerosis has improved slightly (Fig. 4).

The mother originally developed a facial butterfly rash at 20 years of age. At that time, she had Raynaud's phenomenon, arthralgias and photosensitivity. She was diagnosed as having systemic lupus erythematosus, but received no treatment. Six months after delivery, the mother, then 26 years old, again developed a facial rash. She suffered precordial pain and developed a high fever (39°C). An ANF test was positive at a titre of 1:2 560, and anti DNA antibody was present at titre of 1:80. Tests for SSA and SSB antibodies were not performed. RA was 2+ and RAHA gave a titre of 1:5 120. A chest X-ray showed marked cardiomegaly. Two months after the recurrence of SLE she died of cardio-renal failure.

## DISCUSSION

It is recognized that most infants with neonatal lupus erythematosus are born to mothers with connective tissue disorders, such as SLE or Sjögren's syndrome. These infants possess antibodies directed against the cytoplasmic antigens Ro (SSA) and La (SSB), which have a maternal origin and are transferred through the placenta (Miyagawa *et al.*, 1981; Weston *et al.*, 1982; Gawkrodger & Beveridge, 1984; Lee *et al.*, 1983; Diestelmeier, Hayne & Rada, 1984; Lin *et al.*, 1985; Lumpkin *et al.*, 1985). The skin lesions of neonatal lupus erythematosus are characterized by annular erythema, which usually disappears within the first 7 months of life. Generally, maternal immunoglobulins have been cleared by this age. In our case, the annular erythemas which disappeared by the age of 7 months, were considered to be the lesions of neonatal lupus erythematosus. The sclerotic lesions did not clear up within 7 months, and the patient, now 3 years old, still has sclerotic and atrophic lesions on his back, face, chest, shoulder and the nape of his neck.

Three cases of neonatal lupus erythematosus, reported by Lumpkin *et al.* (1985), left significant residual scarring. It is not clear whether the residual scarring of Lumpkin's cases is comparable with the sclerotic lesions of our patient. The atrophic and sclerotic lesions in our patient were much larger than those in Lumpkin's cases. The clinical features and the histology of the three biopsies are compatible with a diagnosis of multiple morphea.

Very few cases of concurrent morphea and DLE or SLE have been reported, and the age of the onset of morphea in these cases was always above 7 years (Umbert & Winkelmann, 1978; Mackel *et al.*, 1979). There are no reported cases of morphea in neonates. Therefore, ours may be the first reported case of concurrent neonatal LE and multiple morphea.

The aetiology of scleroderma remains obscure. There are three theories on the pathogenesis of progressive systemic scleroderma; the vascular theory, the immune theory and the abnormality of collagen metabolism theory (Lee *et al.*, 1984). However, none of these theories is sufficient to explain all of the features of scleroderma. Furthermore, the inter-relationship between PSS and multiple morphea is not clear. It is notable, however, that Umbert and Winkelmann (1978) emphasized the definite relationship between scleroderma and LE, and, particularly, between the two cutaneous forms of these diseases, morphea and DLE.

The sclerotic lesions in our patient may have been caused or initiated by unidentified antibodies, transferred through the placenta from the mother with SLE, for example SSA and SSB antibodies. These antibodies would probably have been cleared within 6–7 months after birth, which would explain why the sclerotic lesions in the patient did not enlarge or new lesions appear after 7 months of age. Generally, once newly formed, collagen fibres cannot disappear easily, whereas the inflammatory change of annular erythema can do so. Atrophic and sclerotic changes in the skin can remain present for a long period.

In the present case, we did not detect SSA and SSB antibodies. However, the patient came to our clinic at 14 months of age, and it was, therefore, probably too late to detect these antibodies. We were not able to test for these antibodies in the mother as she was already dead.

We are continuing long-term follow-up of the patient to monitor the pathogenesis of the scleroderma.

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# Giant solitary trichoepitheliomas located in the perianal area: a report of three cases

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## SUMMARY

Very large solitary trichoepitheliomas which arose in the perianal region in three patients are described. Although these tumours showed a striking histological similarity to classical multiple or solitary trichoepitheliomas of the face, they differed in their massive size, unusual location and by their involvement of deeper tissue.

We suggest that giant solitary trichoepithelioma is a distinct variant of trichoepithelioma that may have a predilection for the perianal region. At this site this rare tumour must be distinguished from basal cell carcinoma of the perineum and from malignant basaloid (cloacogenic) carcinoma of the anal canal.

Two forms of trichoepithelioma are usually recognized, the multiple (epithelioma adenoides cysticum) and the solitary type. They commonly present as small flesh-coloured papules on the face, although occasionally other body sites are affected. Multiple trichoepitheliomas are usually an expression of a familial trait, but in the solitary form there is no family history of the disorder (Gray & Helwig, 1963). Solitary trichoepitheliomas rarely exceed 0·5 cm in diameter and clinically they are often mistaken for small, fleshy, non-pigmented cellular naevi.

Although very few cases of giant solitary trichoepithelioma (GST) have been reported (Czernobilsky, 1972; Filho *et al.*, 1984), a number of histologically similar tumours have been described under the alternative labels of trichoblastoma (Headington, 1970), trichoblastic fibroma (Grouls & Kupfer, 1984) and trichogenic trichoblastoma (Headington & French, 1962; Headington, 1970). These terms were originally coined by Headington (1976) for a variety of large hair follicle neoplasms which are believed to represent complex tumours derived from the epithelial mesenchymal matrix of the hair germ. We believe that these tumours are essentially histologically similar to trichoepitheliomas and we note that Headington (1976) concurs that his trichoblastic fibroma variant closely resembles ordinary trichoepithelioma histologically, except for its deeper location and its greater degree of pseudoencapsulation. For the purpose of

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simplicity we therefore propose retaining the term trichoepithelioma for the large solitary tumours presented in this paper, as clearly histologically these lesions are related very closely to ordinary trichoepitheliomas.

## CASE REPORTS

### *Case 1*

A 77-year-old woman presented to the Dermatology Department at the Royal Free Hospital with a large tumour of the natal cleft which had been present for 7 years and was said to have been enlarging slowly. There was no family history of facial papules or skin tumours.

On examination a soft, pedunculated, smooth, flesh-coloured tumour was found arising at the upper end of the natal cleft in the midline (Fig. 1). A preliminary superficial biopsy taken from the tumour mass showed interlacing lobules of a basaloid tumour embedded in a loose fibromyxoid stroma. A basal cell epithelioma or a form of basaloid hamartoma was suspected. The tumour was excised but the excision was incomplete. The wound healed without complication and the tumour has not recurred 18 months later.

*Pathology*. The gross surgical specimen measured 3·5 cm × 3·5 cm × 2·5 cm. The skin surface was intact except for one area of ulceration. The cut surface of the tumour appeared to be of uniform consistency and was mottled and yellowish-white in colour. Four separate blocks of tissue were taken at right angles to the surface for histological processing; the histological features were similar in each.

Microscopically, the tumour was found to have replaced the dermis and to extend deeply to

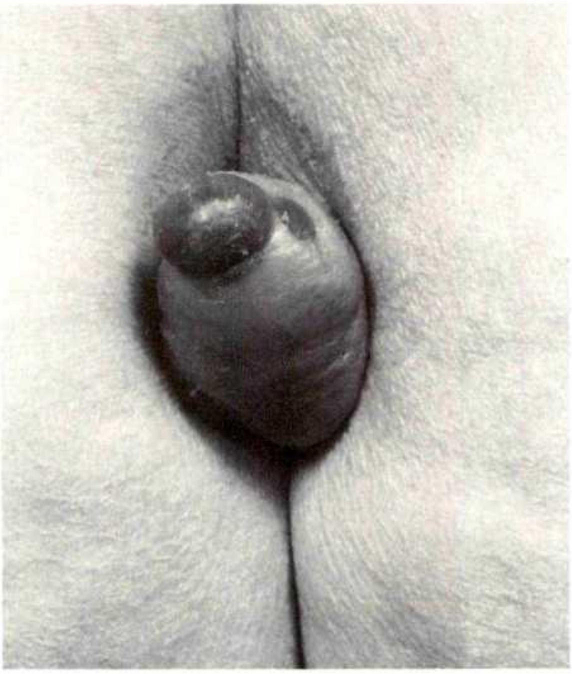

FIGURE 1. Case 1. Pedunculated tumour at natal cleft with a protruberant ulcerated area at upper pole.

involve the fibro-fatty tissue to 2 cm below the skin surface. The tumour was composed of lobules of basaloid epithelium surrounded by a fibromyxoid stroma (Fig. 2). In all the sections, scattered small tumour lobules were present at the level of the papillary dermis, but no direct contact was observed between tumour epithelium and the epidermis. A thin zone of residual upper dermal connective tissue was preserved intact above the tumour, except in the area of ulceration. No encapsulation or compression of connective tissue was observed at the lateral or deep margins of the tumour. Occasional small tumour islands were seen infiltrating the dermis or fat ahead of the main tumour masses. Examination of the tumour at low power showed it to be organized into ill-defined nodules composed of numerous tumour islands. These nodules were separated by areas of loose fibrocellular connective tissue in which tumour islands were sparser (Fig. 2). The larger tumour islands showed a complex pattern with a mosaic arrangement of lobules and strands which were separated by a fibromyxoid stroma. In contrast, the smaller tumour islands tended to occur in closely set grape-like clusters with little intervening stroma (Fig. 2). Occasional longer branching strands of tumour epithelium, two to three cells thick, gave rise to antler-like patterns. The tumour islands and strands were composed of closely-packed cells with prominent nuclei but lacked obvious cell borders and cytoplasmic content. Some palisading of cells at the periphery of the larger cell lobules was found. Dyskeratotic cells or cell atypia was not a feature, but the tumour islands throughout displayed considerable mitotic activity. Only a very occasional tumour lobule showed central squamous metaplasia or incipient horny cyst formation.

A striking feature throughout the tumour was the well-developed myxoid, cell-rich fibrillary stroma (Figs 2 and 3a). The stromal cells possessed prominent ovoid nuclei but had indistinct cell borders; however, generally, the impression was that of spindle or stellate shaped cells. These cells tended to be patterned with their long axes around the tumour lobules. In places condensations of smaller, more rounded stromal cells abutted against or indented the tumour islands (Fig. 3a), giving an appearance suggestive of primitive hair papilla formation. At these sites the adjacent epithelial cells were elongated in a palisaded formation.

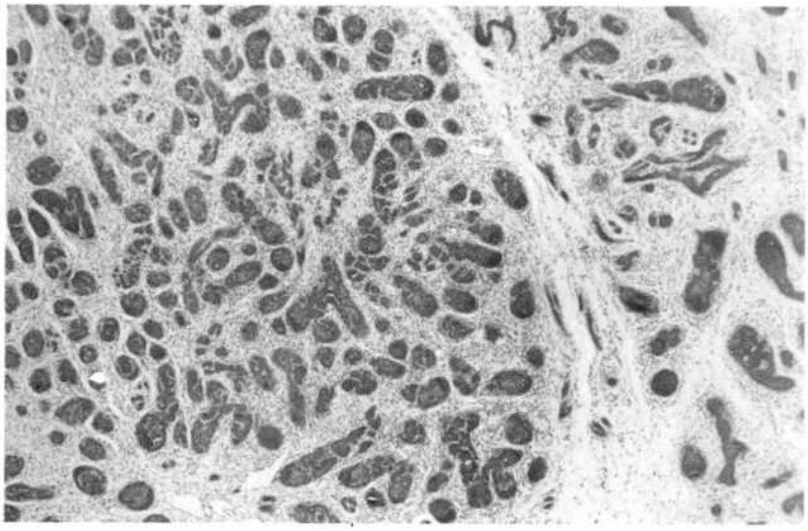

FIGURE 2. Case 1. Tumour islands in a mosaic pattern separated by prominent stromal cells. (H & E, original × 30).

Numerous mast cells were present within the stroma. In one part of the tumour, clumps of amorphous eosinophilic hyaline material suggestive of amyloid were seen (Fig. 3b). The presence of amyloid was confirmed by Congo Red staining and by thioflavin T fluorescence. The sections were also stained with PAS with and without diastase digestion, Gomari's stain for reticulin, orcein van Gieson for elastic tissue, Masson Fontana for melanin and alcian blue stain. PAS staining showed that the stroma surrounding some of the tumour lobules contained areas of hyalinisation. Glycogen accumulation was not a significant feature. The stroma contained acid mucopolysaccharides as demonstrated by the alcian blue method. The reticulin stain showed condensations of reticulin around the epithelial islands, but elastic tissue was sparse.

### *Case 2*

A 71-year-old man presented with a rigor. An incidental finding was a hard mobile tumour measuring 4 cm × 2 cm, situated on the right inner buttock, close to the anal canal. It had been present for many years. The tumour was completely excised and a year later there was no sign of any recurrence.

*Pathology*. The gross surgical specimen comprised an elipse of skin 2·5 cm × 1 cm with an attached underlying smooth mass 5 cm × 3·5 cm × 2·5 cm. On cut section this mass was associated with a thin walled cyst filled with chalky, friable, white material. The cyst extended up to the overlying epidermis.

On microscopy the main tumour was found to be situated deeply in the subcutaneous tissue (Fig. 4a), but two small areas of the tumour were also present, one at the lower border of the dermis, and the other in the upper dermis close to, but not in direct contact with the epidermis. Microscopically, the tumour differed from Case 1 in showing areas with much larger basaloid cell islands, many of which contained keratinous cysts or amorphous areas of cell debris (Fig. 5). However, some smaller cell lobules were also present and these tended to show interlacing

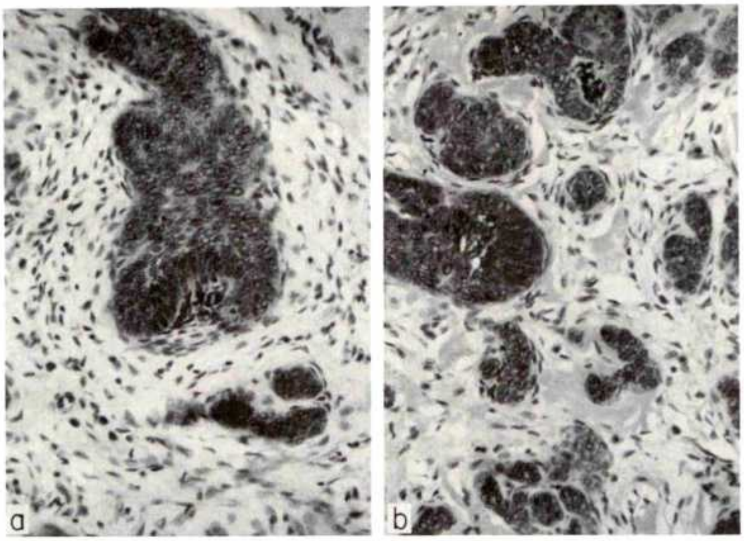

FIGURE 3. Case 1. (a) Hair germ with grouped hair papilla cells; (b) stromal amyloid deposits. (H & E, original × 200).

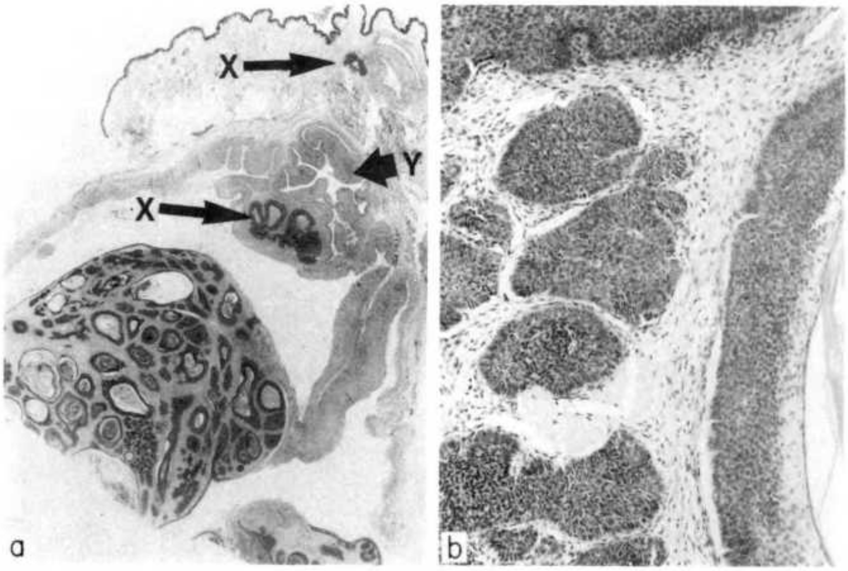

FIGURE 4. Case 2. (a) X: small tumour islands in upper dermis and at lower dermal border; Y: foreign body granuloma around sinus tracts. (H & E, original × 5); (b) probable amyloid deposits at lower left. Prominent stromal cells against tumour epithelium at top. (H & E, original × 80).

communications. A cell-rich mesenchymal fibrocellular stroma similar to that already described in Case 1 invested the tumour lobules (Figs 4b and 5). This stroma showed both loose fibrillary areas, as well as densely collagenous zones with hyalinisation. Also, in places, denser aggregates of stromal cells were seen in close apposition to the tumour epithelium (Fig. 4b). However, hair papilla-like indentations were less prominent than in Case 1. Scattered mitoses were found in many of the tumour lobules. The rupture of a large epidermoid cyst gave rise to an extensive foreign body granulomatous reaction to keratin debris (Fig. 4a). In one area, clumps of eosinophilic hyaline material suggested the presence of amyloid deposits (Fig. 4b), but we were unable to confirm this as spare slides were not available.

### *Case 3*

A 70-year-old woman presented with a large pedunculated nodule, situated in the natal cleft. This tumour had been present for 10 years and was enlarging slowly. The tumour was excised completely and six months later there was no sign of recurrence.

*Pathology*. Macroscopic examination showed a tumour measuring 3·5 cm × 2·0 cm × 2·5 cm and the cut surface was pale and firm. Microscopic examination at low power showed the tumour to have an expansile appearance with the bulk of the tumour elevated above the skin surface (Fig. 6). A narrow band of well vascularized dermal collagen separated the tumour from the epidermis. At higher magnification the tumour was composed of closely set lobules of basaloid cells. Most of the lobules were intermediate in size when compared with those of Cases 1 and 2. There were a few larger lobules, some containing small keratin cysts. Many of the individual lobules were grouped together and showed branching and budding from a central zone. Also, complex patterns of intercommunicating tumour strands gave rise to histological

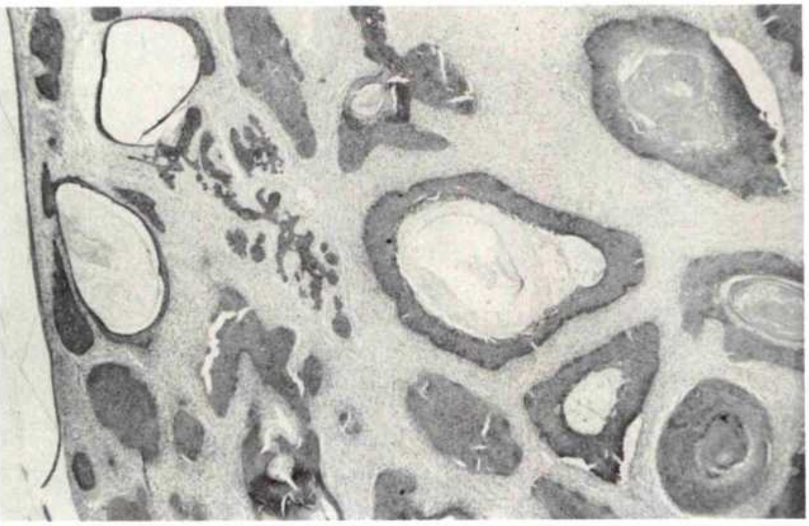

FIGURE 5. Case 2. Tumour islands with keratin cysts, to left tumour strands showing antler-like patterns. (H & E, original × 30).

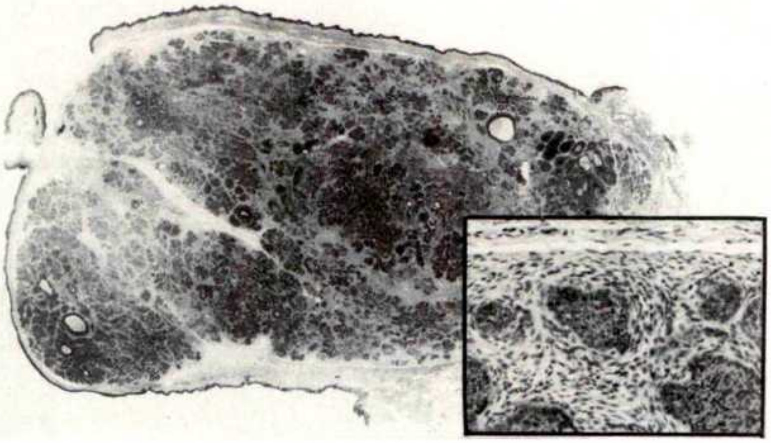

FIGURE 6. Case 3. The main body of the tumour is elevated above the surface of the skin. Note that the tumour is separated from the epidermis by a continuous band of connective tissue. (H & E, original × 5). Inset: margin of tumour demonstrating prominent stromal cells around basaloid lobules. (H & E, original × 80).

patterns similar to those seen commonly in the classical small lesion of trichoepithelioma. Mitotic activity was notable throughout the tumour. A cell-rich fibrillary stroma, similar to that seen in the other cases, accompanied the tumour islands throughout (Fig. 6, inset) and demarcated clearly the tumour from the adjacent dermal tissue and fat. However, the tumour was not encapsulated. Definitive hair germ formation was less in evidence than in Case 1. The stroma contained numerous mast cells. The presence of amyloid was not detected.

## DISCUSSION

GST is a rare tumour as judged by the sparsity of case reports in the literature. We have been able to trace four reports of large tumours which show striking clinical and histological similarities to those reported here; these were variously labelled GST (Czernobilsky, 1972; Filho *et al.*, 1984), trichoblastic fibroma (Grouls & Kupfer, 1984) and trichogenic adnexal tumour (Arata, 1976). In addition, Headington (1970) described three tumours (one trichoblastoma and two trichogenic trichoblastomas) which, in spite of the greater degree of hair follicle differentiation seen in the trichogenic trichoblastoma variant, we believe to be histologically comparable to the GST. By searching the histopathology records at St John's Hospital we have been able to trace three further examples of GST in addition to those described in this report. The clinical features of these six cases, and those of previously reported cases, are detailed in Table 1. Imai & Nitto (1982) have reported an unusual hair follicle tumour under the label trichogenic trichoblastoma, but this tumour showed evidence of sebaceous and apocrine derivation and we have therefore excluded it from our series.

To summarize the data, the 13 tumours were nearly all larger than two centimetres, they usually involved the hypoderm as well as the dermis, and there was no clear sex predominance. GST tends to develop later in life than ordinary trichoepitheliomas. Ten of the tumours listed occurred either in the pelvic girdle region or on or close to the thigh. Only two tumours occurred on the head and neck. In contrast, Headington (personal communication) has informed us that 10 out of the 20 tumours he has collected under the label of trichoblastic fibroma arose on the head and neck. GST is a slow growing tumour that gradually expands and replaces normal tissue, but to date they do not seem to have behaved in a locally malignant fashion and no lesion has been reported to have metastasized.

Except in their overall size and depth of invasion well into the subcutaneous fat, GST shows a striking histopathological similarity to the smaller varieties of trichoepitheliomas. Both share the common features of islands and strands of basaloid epithelium that lack cell atypia,

TABLE 1. Clinical data on 13 cases of giant solitary trichoepithelioma

| Age (years) | | | | | |
|---|---|---|---|---|---|
| At onset | At presentation | Sex | Site | Diagnosis | Reference |
| Puberty | 25 | F | Buttock | Trichogenic trichoblastoma | Headington (1970),<br>Headington & French (1962) |
| ? | 31 | F | Scalp | Trichoblastoma | Headington (1970) |
| ? | 51 | M | Groin | Trichogenic trichoblastoma | Headington (1970) |
| 38 | 58 | M | Thigh | GST | Czernobilsky (1972) |
| 19 | 19 | M | Knee | Trichogenic adnexal tumour | Arata (1976) |
| 18 | 53 | M | Upper thigh | GST | Filho *et al.* (1984) |
| ? | 70 | F | Thigh | Trichoblastic fibroma | Grouls & Kupfer (1984) |
| 70 | 77 | F | Natal cleft | GST | This report |
| ? | 71 | M | Natal cleft | GST | This report |
| 60 | 70 | F | Natal cleft | GST | This report |
| ? | 24 | M | Sacrum | GST | Unpublished cases from the St John's Hospital Histopathology Department |
| 40 | 48 | M | Scalp | GST | |
| ? | 25 | M | Shoulder | GST | |

embedded in a specialized 'organoid' stroma. This stroma is cell rich and is similar to, or analogous with, the specialized connective tissue that encases the mature hair bulb and occupies the dermal hair papillae. Both GST and ordinary trichoepitheliomas may show horny cyst formation and foci where there is an impression of hair germ induction. In both, complex interlacing or branching antler-like patterns of tumour epithelium are common and are a distinguishing feature from basal cell carcinoma. The presence of numerous mast cells within the stroma was noted in Cases 1 and 3. This feature has been demonstrated in multiple and solitary trichoepithelioma and also in basal cell carcinoma (Gray & Helwig, 1963). The main differences between GST and ordinary small trichoepitheliomas lie in the much greater degree of mitotic activity in GST and their wider range of histological expression. In particular, in Case 2 many of the tumour lobules were very large with correspondingly large horny cyst formation. The definite and suggested presence of amyloid in two of our cases (Cases 1 and 2 respectively) may also be a further minor distinguishing point from other trichoepitheliomas. Amyloid deposits are found quite commonly in basal cell carcinoma and a variety of other skin tumours (Runne & Orfanos, 1977), thus their presence in GST is not diagnostic. In none of the six tumours we have studied have we encountered hair matrix differentiation to the degree found in trichofolliculoma (i.e. formation or incipient formation of hair shafts). Such advanced morphological differentiation was encountered in the two large tumours described by Headington and French (1962) and Headington (1970) (*see* Table 1) under the label of trichogenic trichoblastoma. 'Pseudo-encapsulation' mentioned by Headington (1976) as occurring around lesions of trichoblastic fibroma was inconspicuous in the cases of GST that we have studied.

In the detailed classification of hair follicle tumours by Headington (1976), ordinary trichoepitheliomas are listed under the general heading '*Hamartomas* of the hair germ', while trichoblastic fibromas and trichogenic trichoblastomas are listed as 'Epithelial trichogenic *tumours* with induction'. We agree with the view that ordinary trichoepitheliomas are essentially small stable hamartomatous or organoid lesions, whereas in GST the slow progressive growth, the mitotic activity and the later age of onset suggest a new growth or neoplasm, albeit benign in nature. Thus, we consider that GST is the benign tumour equivalent of small organoid trichoepitheliomas. To avoid confusion, we do not consider that there is any need to coin a new name for GST.

The peculiar perianal distribution of these three GSTs raises the question of the differential diagnosis of these tumours from other basal cell tumours which may occur in this area. Basal cell carcinomas may develop rarely in the pudendal region and natal cleft, particularly in the elderly (Nielson & Jensen, 1981; Kraus, 1978). These carcinomas arise directly from the epidermis and they show no essential histological difference from other basal cell carcinomas which arise at the more usual sites. In particular, they do not show a patterned 'organoid' stroma or evidence of hair germ induction. The basaloid carcinoma of the anal canal (Pang & Morson, 1967) arises well within the anus, at or above the dentate line, from non-keratinizing squamous or transitional epithelium. Despite the structural similarity of this tumour to ordinary basal cell carcinoma of the skin, it is an aggressive tumour capable of metastasis. Its site of origin, its poorly differentiated cytological appearance and its lack of organoid features should serve to distinguish this tumour from GST.

We hope, by this report, to draw attention to the rare large solitary type of trichoepithelioma, a tumour that, to date, has been poorly documented in the literature. We suggest that this tumour may have a predilection for the pelvic girdle and thigh area, and more particularly the perianal region. At this latter site, GST must be differentiated from other more aggressive basaloid tumours that occur in this area.

## ACKNOWLEDGMENT

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# Microcystic adnexal carcinoma

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## SUMMARY

A patient is described with a tumour of the upper lip which was proved histologically to be a microcystic adnexal carcinoma, probably derived from the outer hair root sheath. This appears to be the eighth in the literature.

Microcystic adnexal carcinoma (MAC) is an unusual form of cutaneous carcinoma which has been described recently by Goldstein, Barr and Santa Cruz (1982). The tumour affects predominantly the upper lip of young and middle-aged women, with repeated local recurrence leading to devastation of the face. No cases of metastasis have been reported to date.

We describe a case of MAC which, to the best of our knowledge, is the eighth in the literature and appears to fulfill completely the diagnostic criteria of this neoplasm.

## CASE REPORT

A 29-year-old woman presented with a tumour on her upper lip, which had appeared 18 months earlier. It had been treated by superficial electrodessiccation but had recurred a few months later.

On examination, the tumour was a 1 cm, flesh-coloured nodule, with a smooth, shiny surface, small telangiectases and a central smaller pedunculate nodule. The latter nodule raised some doubts regarding the otherwise obvious clinical diagnosis of basal cell carcinoma, and was removed for histology, which resulted in a tentative diagnosis of 'unusual adnexal epithelioma'. Therefore, the remainder of the tumour (Fig. 1) was excised.

### *Histopathological observations*

At scanning magnification, the tumour appeared to involve the whole dermis, infiltrating the subcutis and the first lines of the cells of the orbicularis oris muscle (Fig. 2). Although the tumour mass was compressing both epidermis and adnexa, no connections to them were apparent.

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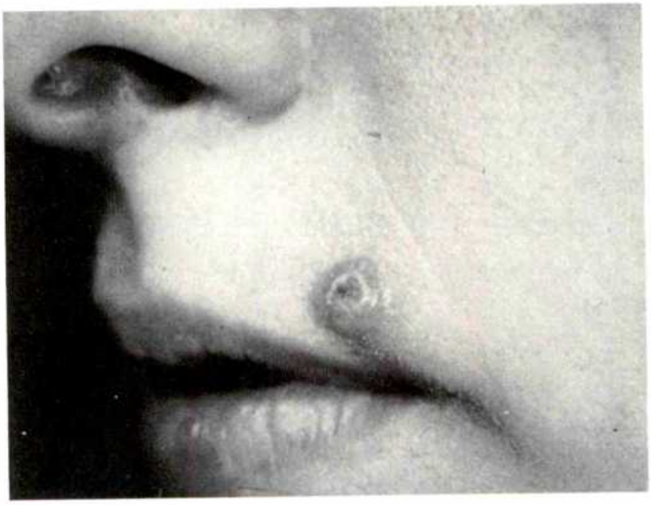

FIGURE 1. The tumour on the upper lip.

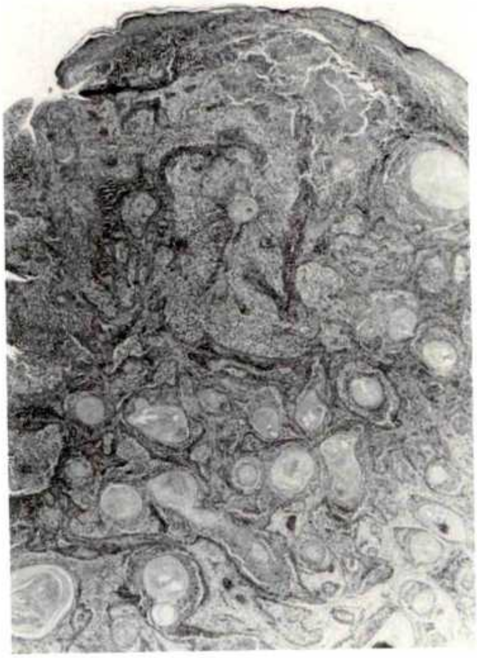

FIGURE 2. Numerous keratin-filled cysts occupy the whole dermis.

The tumour consisted of cellular islands or nests of various sizes, most of which included keratin-filled cysts. The keratin in the cysts was either compact or laminated and, in some instances, formed onion-like globes (Fig. 3). The cellular nests were embedded in a fibrous stroma containing inflammatory cells. No dermal retractions, of the type observed in basal cell carcinoma, were visible.

At higher magnification, the cellular nests appeared to consist of a layer of basaloid palisading

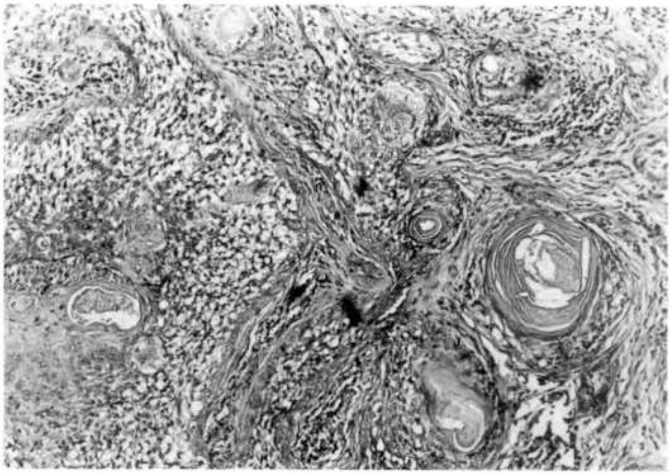

FIGURE 3. Cellular islands of various sizes including keratin-filled cysts and onion-like globes.

cells at the periphery and, in the centre, a layer of polygonal cells with a clear cytoplasm and eosinophilic cells forming keratinic cysts and horn globes (Fig. 4).

Cellular atypia and mitotic figures were rare, but the tumour had infiltrated the deeper corium, the skeletal muscle and the perineural spaces. In the deeper parts, most of the cellular nests had no horn cysts and showed a trabecular pattern. Although several sections were cut, neither tubular nor alveolar structures were observed.

A PAS-positive diastase-sensitive material was demonstrated in the clear cells and was interpreted as being glycogen. Rhodamine B (Hanau, Marchand & Grosshans, 1980) showed that keratin in the horn globes and in the cysts was epidermoidal. Staining with Alcian blue disclosed notable PAS-negative mucin deposits in the deep dermis, consisting of hyaluronic acid.

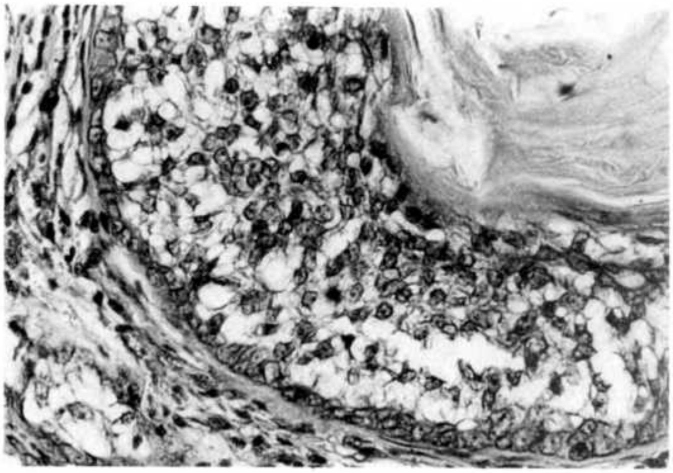

FIGURE 4. The islands are composed of basaloid palisading cells and of polygonal clear cells in the middle, resembling cells from the outer hair root sheath.

Immunohistochemical studies using the peroxidase-antiperoxidase technique for CEA yielded negative results, whereas a polyclonal anti-keratin antibody identified keratin in the tumour cells and in the cysts.

A diagnosis of MAC was made and the patient submitted to a larger and deeper excision.

## COMMENT

The tumour in our patient fulfills the diagnostic criteria of Goldstein *et al.* (1982) for MAC, namely: (i) multiple cellular nests, most of which form keratin-filled cysts; (ii) infiltration of subcutis and skeletal muscle; (iii) a fibrous stroma sometimes rich in mucin; and (iv) permeation of perineural spaces.

MAC is a distinctive clinico-pathological entity with a spectrum of histological features, including tumours with glandular or pilar differentiation, or both. In this context, our case is similar to the tumour described by Cooper and Mills (1984) in that it showed glycogen-rich clear cells and neither tubular nor alveolar structures. In addition, it exhibited a well-recognizable follicular differentiation reminiscent of the outer follicular sheath. Such a feature, although not mentioned by the authors, may be recognized also in the tumour described by Cooper and Mills (1984).

With regard to histogenesis, the histological and histochemical features of our case point to a keratinizing follicular structure and not to a glandular one, suggesting an origin in the primary epithelial germ, or directly from the outer root sheath.

## ACKNOWLEDGMENT

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# Atrophia maculosa varioliformis cutis

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## SUMMARY

We describe a case of atrophia maculosa varioliformis cutis (AMVC), a rare form of idiopathic facial macular atrophy. A biopsy revealed only a depression in the epidermis, probably caused by loss of dermal collagen. Because AMVC may be confused with scarring, and factitial disease may be suggested, it is important that this condition be recognized by the physician.

Macular atrophy refers to circumscribed areas of soft, depressed and sometimes finely wrinkled skin, often characterized histologically by deficiency of dermal connective tissue (Cunliffe, 1979). Clinically, the skin appears to be thin. Primary macular atrophies are idiopathic, while secondary macular atrophies result from destruction of dermal connective tissue in patients with known inflammatory disorders. Macular atrophy can result from loss or destruction of collagen or elastic tissue in the dermis. Heidingsfeld (1918) described a rare form of limited, non-inflammatory macular atrophy termed atrophia maculosa varioliformis cutis.

## CASE REPORT

A 22-year-old Caucasian male college student was referred to the Geisinger Clinic in January 1984, for evaluation of unusual facial scarring. In September 1982, the patient had noticed some 'marks' on his cheeks near the mouth angles. Over the next 16 months, he developed many new asymptomatic areas without preceding inflammation. He reported that a 'new depression' in the skin would suddenly appear and then increase in size linearly over a few days or weeks. He denied any trauma to his face. He had had very mild acne as a teenager but denied any problem with acne for the past few years. His weight had not changed significantly. Medications included alprazolam (Xanax) and trazadone hydrochloride (Desyrel) since January 1983, for depression. In 1980, he had had testicular surgery for an undescended left testicle. The patient was otherwise fit except for mild low back pain and subjective hearing loss which he attributed to loud noise exposure during military service. Family history was significant. An 18-year-old brother and a 19-year-old sister had recently developed similar facial changes. We examined the brother and confirmed these changes. A 10-year-old brother and the parents were reportedly unaffected.

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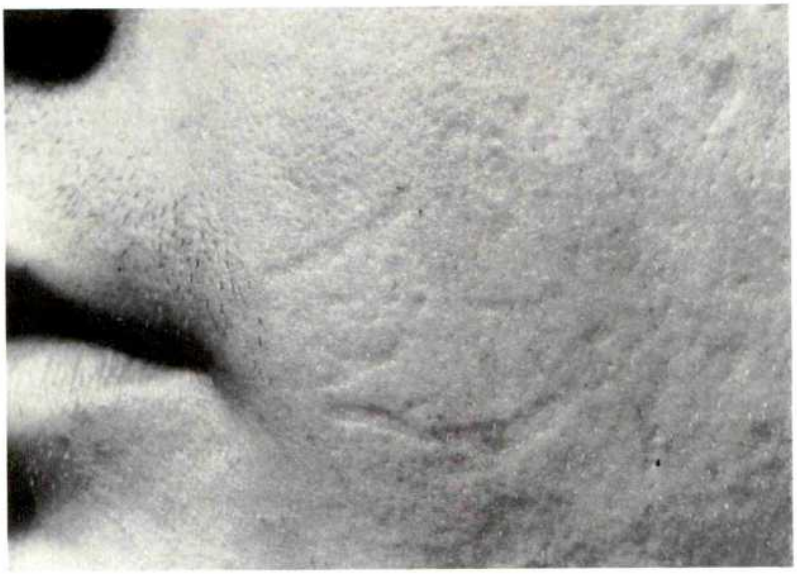

FIGURE 1. Patient's cheek showing a number of linear depressed lesions.

Examination of our patient revealed very discrete facial depressions, mostly linear, with a tendency to radiate onto the cheeks from the area immediately lateral to the mouth angles (Fig. 1). Similar changes were noted along the inferior and lateral aspects of the angles and rami of the mandibles. The atrophic sites varied from 4 to 15 mm in length, and were approximately 2 mm wide. Several 3–5 mm varioliform changes were present on the superior, lateral cheeks. The margins of these changes were very sharply defined with abrupt, almost perpendicular,

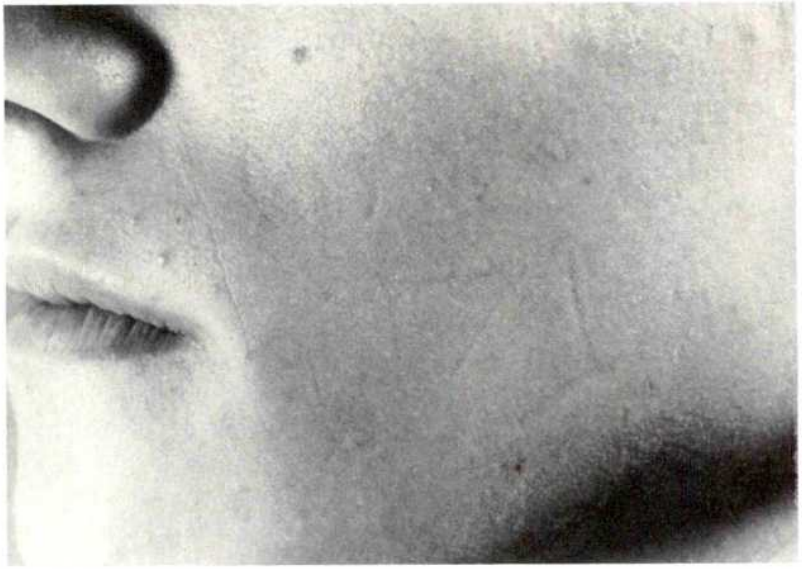

FIGURE 2. Patient's brother showing similar but less marked changes.

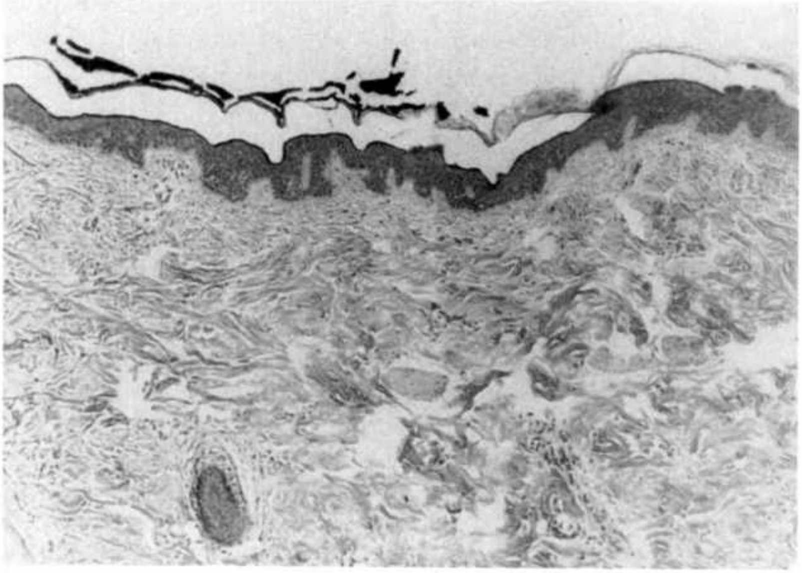

FIGURE 3. Low power view of lesion showing slight depression. Dark-stained stratum corneum indicates area of lesion.

'drop-offs' from the surrounding skin to a depth of approximately 1 mm. The sites were soft, of normal skin colour, and without scale. The remainder of the skin and general physical examination were normal except for the absent left testicle. The 18-year-old brother had similar but fewer changes on both cheeks (Fig. 2).

A 5 mm punch biopsy was taken from a typical 2 mm wide lesion and adjacent normal skin

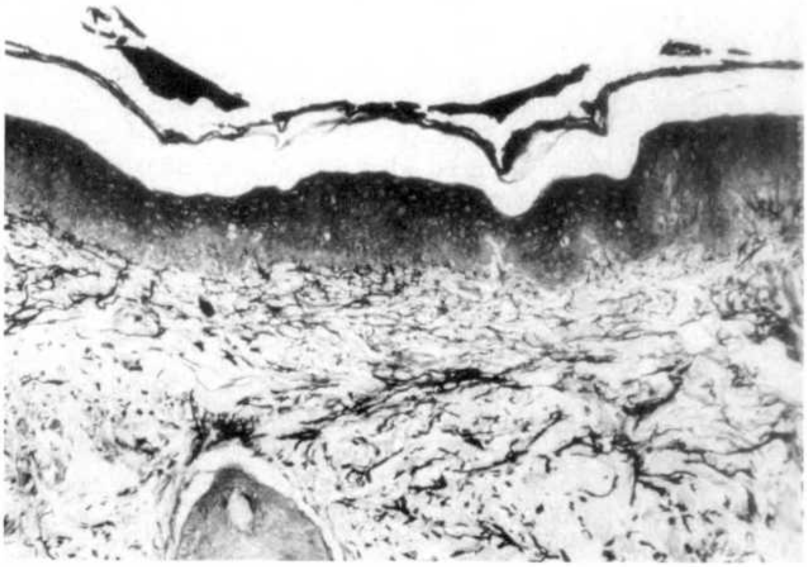

FIGURE 4. Higher power view of same area as illustrated in Figure 3. Van Gieson stain shows normal elastic fibre pattern of staining.

near the angle of the mandible. In an attempt to identify precisely the margins of clinical change, the area was marked before biopsy with a ball point pen and marked again with India ink before processing.

### *Histology*

Only a slight depression of the stratum corneum and epidermis could be seen where the surface of the lesion had been marked with India ink (Fig. 3). Stratum corneum, epidermis and dermis were normal. The papillary dermis appeared to be the same thickness throughout the biopsy specimen and no difference could be detected in the thickness, orientation or staining properties of individual collagen bundles. Overall dermal thickness could not be measured accurately, although no difference could be seen between lesional and perilesional areas. A normal hair follicle coursed through the dermis beneath the area marked as lesional skin. Sweat glands were normal. With Verhoeff van Gieson stain, elastic fibres appeared normal in all areas of the biopsy (Fig. 4). Reticulin fibres showed a normal pattern. There was no evidence of an inflammatory infiltrate or fibrosis.

## DISCUSSION

Our patient and his brother presented with a distinctive clinical picture of circumscribed atrophy. Three cases of AMVC have been reported previously in the literature.

Heidingsfeld's original report described a 20-year-old man with changes limited to the face. Many were indistinguishable from the 'pit marks' following a variola infection, although some of the lesions were linear and rectangular. Faint erythema and scale reportedly preceded the development of the atrophic lesions. Senear (1923) described an 18-year-old woman with pitted facial scars unrelated to acne and not preceded by inflammation. McCorriston and Roys (1951) reported a 37-year-old woman with facial pits which had begun to form at the age of 35. She had varioliform, linear and rectangular changes described as 'shallow, atrophic scars' present on her cheeks. At times these asymptomatic lesions were preceded by minute areas of erythema and scaling which allowed the patient to predict the site of development of new lesions. No biopsies were performed on any of these cases, and family histories were not mentioned.

In contrast to the predominance of varioliform changes in the previous cases, our patients displayed mostly linear and rectangular depressions with only a few varioliform pits. On casual examination, the changes of AMVC might be confused with scars. However, in contrast to scars, AMVC is characterized by sharply defined edges almost perpendicular to the surface, and absence of erythema, induration or colour variation. The areas are shallow and are best examined by side lighting rather than by palpation. Scale is absent.

The radiation of the changes from the mouth angles in our patient and in his brother created a bilateral, somewhat symmetrical, appearance. Although not invariable, the atrophy tended to follow Langer's lines. Newer lesions were exactly the same skin colour as the older lesions. No inflammation was noted at any stage, clinically or histologically.

The aetiology of this clinical entity has not been identified. All the previously reported cases were sporadic. Family clustering in this case suggests an inherited disorder or a common environmental insult. If inherited, then the occurrence only in siblings, the involvement of both sexes, and the apparent rarity would favour an autosomal recessive mode of inheritance. Conceivably, the atrophy could develop in response to an infectious agent or an absorbed toxin, with point source exposure accounting for familial clustering. Direct external injury would seem unlikely, given the symmetry of the changes, involvement only of the face in all cases, and lack of any demonstrable histopathological changes.

Nosologically, it seems appropriate to group this disorder with the macular atrophies because of the clinical impression of a circumscribed decrease in dermal thickness. The term anetoderma does not apply, however. Anetoderma implies a loose or flaccid appearance. According to Venencie and Winkelmann (1984), the criteria for anetoderma include circumscribed areas of slack skin with a characteristic herniation on palpation of lesions, the sensation of loss of dermal substance, and the histological loss of elastic tissue in the mid-dermis. The lesions of the Jadassohn-Pellizari and Schweninger-Buzzi types of primary macular atrophy are flaccid and often consist of bladder-like protrusions which yield on pressure through a defect with a surrounding ring of normal skin. Histologically, dermal elastic tissue is fragmented. Neither loose, flaccid, bladder-like protrusions nor histological fragmentation of dermal elastic tissue are seen in AMVC. The only histological change seen on our biopsy was a depression of the epidermis in the area of the clinical depression. Since the epidermal thickness was uniform, one must assume that the depression resulted from a subtle loss of dermal connective tissue. With normal elastic tissue and appendages, a diminution in collagen is presumed but not proven because of our lack of clinically separable criteria and histological studies.

It seems reasonable to separate and classify the atrophic disorders on the basis of the structural cause of the atrophy, i.e., collagen or elastic tissue defect. Randle and Muller (1983) have classified extensively those conditions caused by or demonstrating elastolysis. Histological staining of elastic tissue makes such conditions definable. Defects or decreases in collagen, however, can be quite subtle histologically. The differential diagnosis of such focal dermal atrophies was listed by Venencie and Winkelmann (1984) and includes scars, morphea, lichen sclerosus et atrophicus, atrophoderma of Pasini and Pierini, perifollicular atrophoderma, perifollicular macular atrophy, striae distensae, and AMVC. Diminution or disruption of dermal collagen can be marked or subtle in all of these conditions. We believe AMVC should be included in the list of those conditions with a decrease in dermal collagen.

Although the aetiology is unknown and the nosology is, at the present time, somewhat arbitrary, the physician must recognize this rare and distinctive clinical entity. Our patient was distraught over the repeated questioning about the cause of the scarring. The patient's underlying depression reinforced the physician's suspicions of covert, external trauma. Awareness and diagnosis of this obscure entity can be followed by reassurance of the patient and avoidance of a psychologically traumatic medical investigation.

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*British Journal of Dermatology* (1986) **115**, 111–116.

# Three cases of eccrine porocarcinoma

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## SUMMARY

We report three cases of eccrine poroma, present for many years, in which features were seen suggesting transformation from a benign to a malignant tumour. These changes ranged from *in situ* Bowenoid dysplasia to frankly invasive squamous carcinoma. The most helpful diagnostic feature in distinguishing the origin of the tumours was the presence of strong cytoplasmic staining for carcinoembryonic antigen (CEA) in cells surrounding, and giving rise to, neoplastic ducts and clefts. Dermatopathologists examining eccrine poromata should examine the lesions carefully for any evidence of malignant change.

Eccrine poromata are thought to arise from the acrosyringium, the intra-epidermal portion of the sweat duct (Hashimoto & Lever, 1964). They are reported to occur most commonly on the foot, although they can be found anywhere on the body (Hyman & Brownstein, 1969). Histochemically, these tumours show evidence of active glycogen metabolism contrasting with the surrounding epidermis (Sanderson & Ryan, 1963). Malignant eccrine poroma (porocarcinoma) was first described by Pinkus & Mehregan (1963). Subsequent reports of porocarcinoma have described malignant change in lesions of long duration (Shaw *et al.*, 1982). The potential of these tumours for metastasis and local recurrence which may be resistant to therapy has been emphasized (Gschnait *et al.*, 1980; Darley, Pegum & Pollock, 1983).

## CASE REPORTS

### *Case 1*

A 77-year-old male presented with an asymptomatic lesion which had been present for 9 years on the thigh and which was slowly increasing in size. It was excised during a laparotomy for a well-differentiated adenocarcinoma of the caecum. The lesion was noted to be sessile, polypoid, and rather livid in appearance and measured 10 mm in diameter at the time of excision.

On section, the tumour was white and firm and contained small irregular cystic spaces. Histologically, it comprised trabeculae of small regular squamous cells showing multifocal connections with the overlying epidermis. On the deep aspect the tumour extended down to the subcutis. Throughout the tumour there were irregular clefts and spaces. These features of eccrine poroma were complicated by foci of severe Bowenoid dysplasia (Fig. 1). There was no

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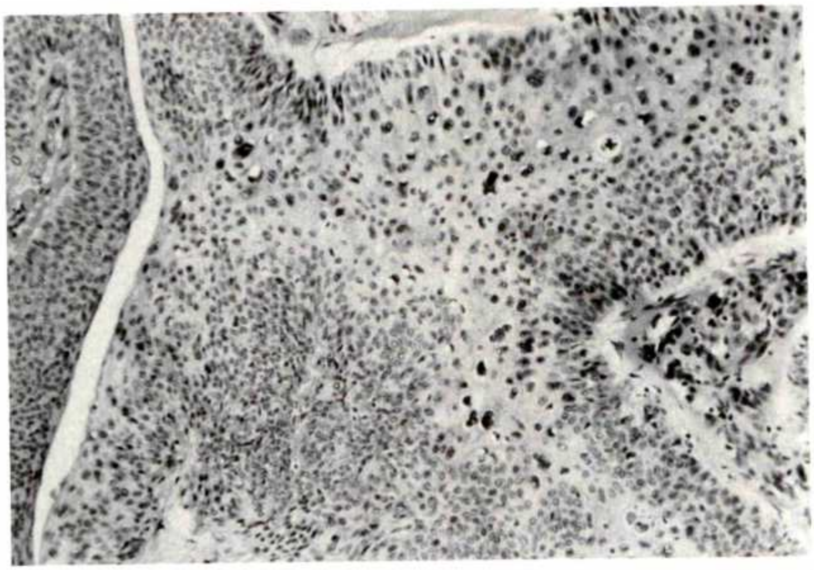

FIGURE 1. Case 1. Photomicrograph of the centre of the tumour. A tumour cleft passes vertically down the left side of the field surrounded by regular epithelium. In the upper centre is a focus of nuclear pleomorphism and hyperchromasia with abnormal mitotic figures. (H & E; original × 200).

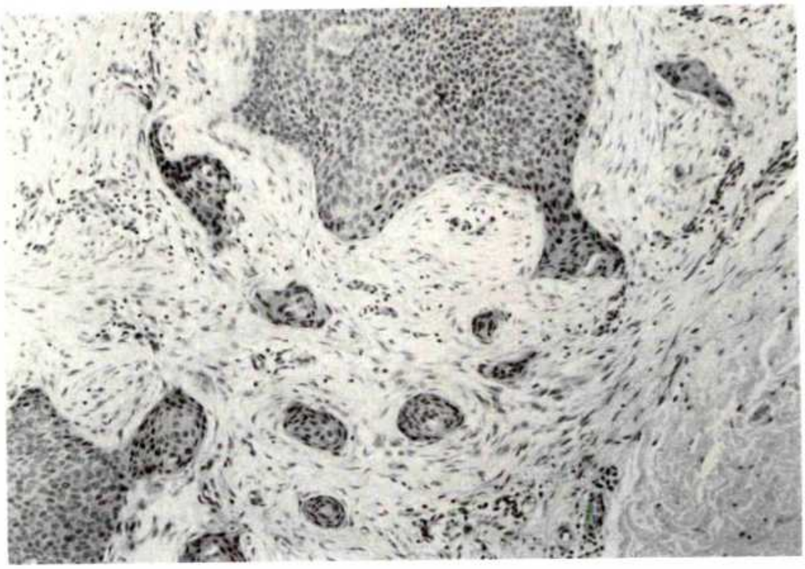

FIGURE 2. Case 1. At the edge of the tumour, small islands of tumour are surrounded by fibrous collagenous stroma arranged concentrically. The appearance is reminiscent of basal cell carcinoma. (H & E; original × 200).

evidence of malignant dermal invasion but, at the lateral dermal margins of the lesion, nests of tumour cells were associated with concentric fibrosis similar to the stromal reaction seen in invasive basal cell carcinomata (Fig. 2).

Follow-up of this patient over 4 years has shown no evidence of recurrence.

### *Case 2*

An 84-year-old female presented with a soft pedunculated lesion on the scalp. The tumour had been present for 20 years, and she had sought help because of ulceration and discharge from the lesion. She had a past history of maturity-onset diabetes and pernicious anaemia.

The tumour measured 50 mm in diameter and was purple with superficial erosion and crusting which was attributed initially to infection of the lesion. A biopsy was performed in 1978 and the histological appearances were those of eccrine poroma.

The ulceration and exudation did not respond to antibiotic therapy and excision was undertaken 6 months later. At operation, the lesion appeared to be raised above the normal skin surface with no deep infiltration. The cut surface was white with cystic clefts and focal haemorrhage. Histological examination showed appearances of eccrine poroma. However, in many areas there was extensive severe Bowenoid dysplasia, with focal malignant stromal invasion (Fig. 3). There was no evidence of invasive growth beyond the boundaries of the main tumour mass.

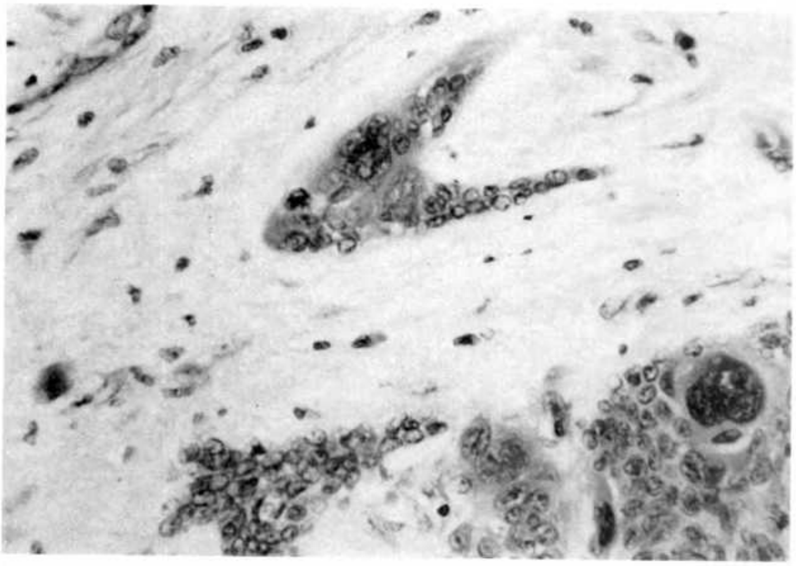

FIGURE 3. Case 2. Bowenoid dysplasia and stromal invasion. (H & E; original × 500).

Follow-up showed no evidence of recurrence prior to her death 16 months later following a laparotomy for an appendix abscess. Post-mortem examination was not requested.

### *Case 3*

A 64-year-old male presented in 1982 with two lesions on the dorsum of his foot. One of the lesions had been present for 20 years, the other had arisen adjacent to it 10 years later. They were raised, plaque-like lesions with a rather warty brown surface. The smaller one was excised and histology showed epidermal acanthosis and hyperkeratosis, with multiple intra-epidermal nests of small, regular squamous cells along its length. There was no evidence of any dermal component. The appearances thus corresponded to hidroacanthoma simplex, the intra-epidermal variant of eccrine poroma. The additional presence of dyskeratotic cells and occasional mitotic figures was interpreted as evidence of dysplasia. The patient had been treated previously for Hodgkin's disease.

The patient failed follow-up for 1 year; when reviewed, the remaining lesion was more fleshy and erythematous, measuring 20 mm × 15 mm × 3 mm. The surface was lobular and focally ulcerated. Excision of the lesion and skin grafting were performed. Histological examination of this tumour showed a residual intra-epidermal component together with trabeculae of tumour tissue arising from the epidermis and extending into the upper dermis. This dermal component contained clefts and ductal structures. In addition to these features of eccrine poroma, there was a focus of invasive squamous carcinoma associated with epidermal ulceration (Fig. 4). The tumour was excised completely.

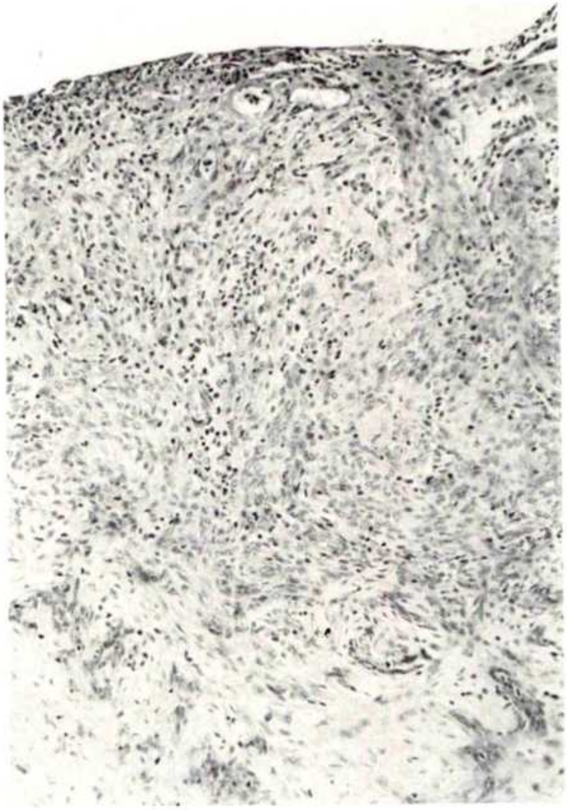

FIGURE 4. Case 3. Inflammation and ulceration associated with a focus of infiltrating carcinoma. (H & E; original × 200).

Follow-up of this patient over 7 months has shown no evidence of recurrence or metastasis.

### *Histochemistry and immunocytochemistry*

All of these tumours showed diastase labile PAS-positive granules within the cytoplasm representing glycogen storage. The ducts and clefts were both lined by and contained diastase-resistant PAS staining cells and debris. The intra-epidermal nests in Case 3 showed no PAS-positive encapsulation. In Case 3 evidence of glycogen metabolism was demonstrated by the presence of strong cytoplasmic staining within the tumour for amylophosphorylase and for succinic dehydrogenase. This contrasted sharply with the negative staining of the surrounding epidermis.

Carcinoembryonic antigen (CEA) is found in the secretory cells and ducts of eccrine and apocrine sweat glands (Penneys, Nadji & Morales, 1982a). In addition, Penneys *et al.* (1982b) also described strong CEA staining in four skin carcinomata of sweat gland origin. One of these tumours showed mixed features of malignant eccrine poroma and clear-cell hidradenoma and showed extensive staining of cells lining the duct-like formations and luminal spaces within the tumour. However, Heyderman *et al.* (1984) were unable to demonstrate any CEA staining in 16 malignant eccrine poromata. These differences are difficult to explain but may relate to differing specificities and degrees of purification of the antisera used by different groups.

We used a polyclonal antiserum to CEA (DAKO Ltd, High Wycombe, Bucks, U.K.) without affinity purification, but with appropriate histological controls in an indirect immunoperoxidase technique. In each of the three tumours reported here, CEA staining was demonstrated by the indirect immunoperoxidase technique in the cells lining the neoplastic clefts and ducts. It was also possible to demonstrate foci of cells containing cytoplasmic vacuoles staining with CEA (Fig. 5). These are interpreted as light microscopic evidence of the mode of formation of neoplastic ducts in these tumours previously demonstrated by electron microscopy. Fusion of intracytoplasmic vacuoles of adjacent cells is thought to recapitulate the embryonic development of the acrosyringium (Hashimoto & Lever, 1964).

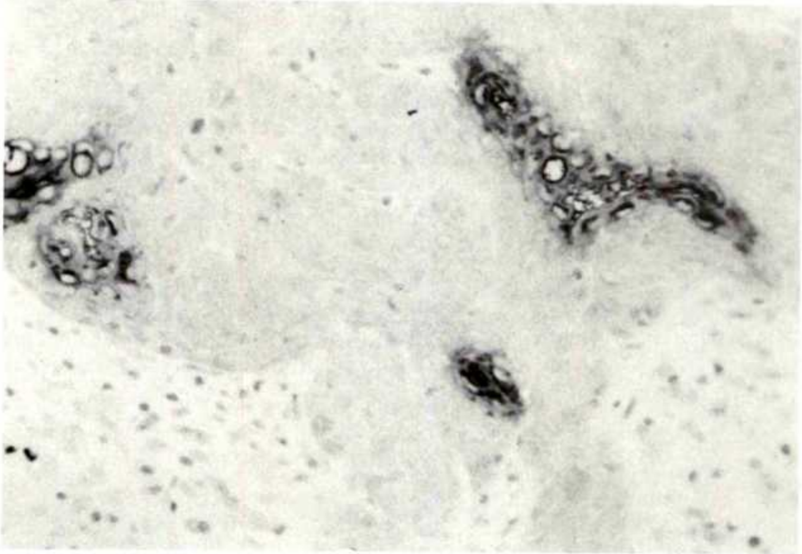

FIGURE 5. Case 3. CEA demonstrated in the cell lining and giving rise to ducts within the tumour. The irregularity of the lumina and the presence of branching exclude the incorporation of normal sweat ducts. (Immunoperoxidase, original × 500).

## DISCUSSION

In each of these three cases, the tumours showed features of dysplasia, or of varying degrees of stromal or dermal invasion within lesions retaining the features of eccrine poromata. All three lesions had been present for many years. Thus, it seems probable that malignant change may supervene in these otherwise benign lesions as a function of age. It is of interest to note that the first lesion excised in Case 3, whilst of a known duration of 10 years, retained the architecture of an intra-epidermal lesion but showed cytological evidence of dysplasia. Bardach (1978) has also reported a single case of hidroacanthoma simplex showing progression to '*in situ* porocarcinoma'. This lesion was present for 15–20 years and showed focal cytological evidence of dysplasia without frank invasion of the dermis. All of these features are very similar to the initial biopsy in Case 3 reported here. We feel that it is important to recognize this possibility in eccrine poromata of long duration and to examine the tumours carefully to exclude invasive malignancy.

## ACKNOWLEDGMENTS

We are grateful to Mr H. Brown, Professor I.D.A.Johnson and Dr G.Holti for permission to report on their cases, and to Dr H.A.Ellis for drawing our attention to Cases 1 and 2, and for histological advice. Histochemistry and immunocytochemistry were kindly performed for us by Mr F.Carpenter.

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### Subcorneal pustular dermatosis followed by pyoderma gangrenosum

MADAM, We were interested to read the recent case report by Marsden and Millard (*British Journal of Dermatology* 1986, **114,** 125) of pyoderma gangrenosum, subcorneal pustular dermatosis and IgA paraproteinaemia. We have recently seen a remarkably similar patient.

In 1979, a 59-year-old woman presented with a 6-month history of a widespread burning eruption. Examination revealed 1 cm flaccid, pus-filled blisters (Fig. 1a) and multiple superficial erosions, measuring 1–5 cm in diameter (Fig. 1b). Some of these erosions were crusted, while others showed central clearing with a scaling serpiginous border, surrounded by a halo of erythema. Both bullae and erosions were widely scattered but showed a predilection for the flexural areas, including the groin, axillae and submammary region; the face was spared. Healing occurred slowly without scarring but left very persistent pigmented marks.

Investigations showed a slight neutropenia (WBC: $3{\cdot}7 \times 10^9$/l; 44% polymorphs) and an ESR of 55 mm/hour, but were otherwise normal. Immunoglobulins were not measured. Biopsies of the lesions showed superficial pustules covered by a thin layer of keratin with occasional acantholytic cells. Polymorph neutrophils were seen infiltrating both the dermis and epidermis, singly and in groups. Direct immunofluorescence was negative on two occasions.

A diagnosis of subcorneal pustular dermatosis of Sneddon-Wilkinson was made. The patient declined treatment and was lost to follow-up until 6 years later when she was re-admitted with a rapidly enlarging ulcer above the lateral malleolus, regarded as typical of pyoderma gangrenosum (Fig. 2). The pustular dermatosis had been unremitting over the course of 6 years and many typical lesions were present. A biopsy from the ulcer showed areas of dermal haemorrhage and abscess formation associated with neutrophilic polymorphs and surrounding fibrosis. No micro-organisms were recognized.

The full blood count and differential white count were normal but the ESR was still raised to 55 mm/hour and the immunoglobulin estimation showed an elevated IgA concentration of 7·7 g/l (normal 0·8–5·0 g/l). Both IgG and IgM were normal. Immunoelectrophoresis showed a small serum monoclonal IgA band. Urinary paraproteins and Bence-Jones proteins were not detected. A bone marrow investigation was not done, and a decision was made to observe the paraprotein concentration over a period of time. The

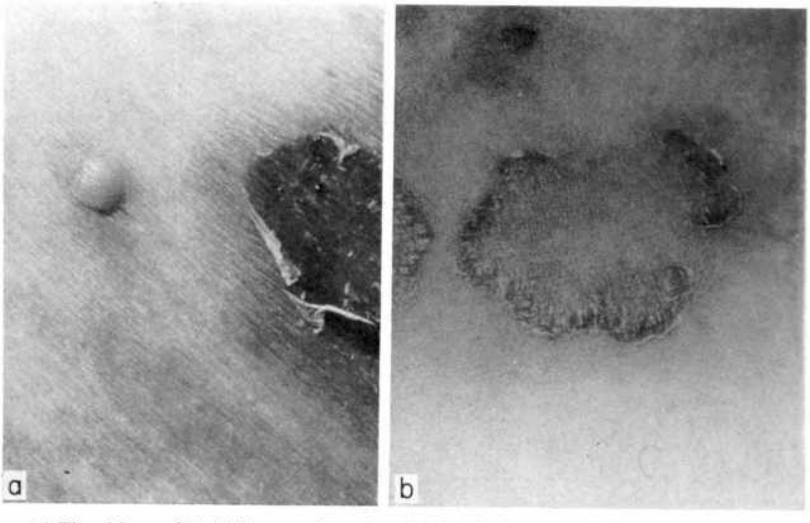

FIGURE 1. (a) Flaccid pus-filled blister and erosion. (b) Serpiginous eroded lesions with scaling edge and central healing.

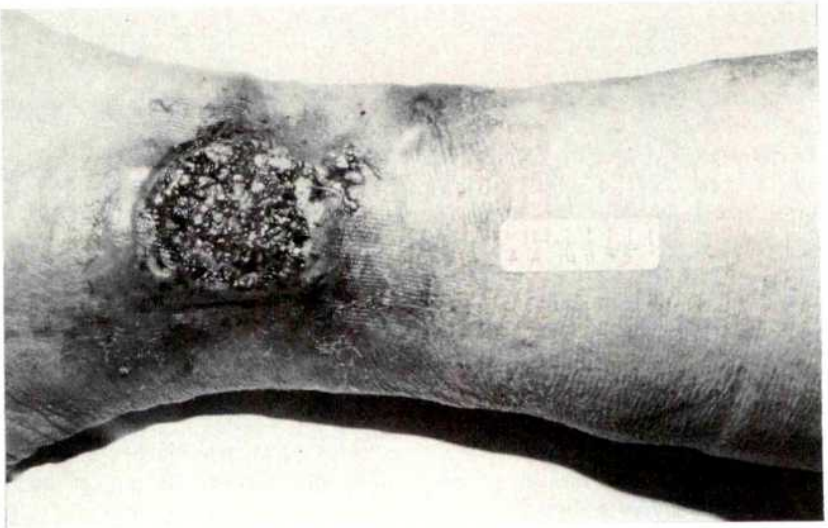

FIGURE 2. Pyoderma gangrenosum.

patient was treated with dapsone 100 mg daily and Dermovate NN® ointment applied to the pyoderma, as well as conventional dressings and elevation of the leg. The pustular and eroded lesions remitted on this treatment, and at recent follow-up the pyoderma gangrenosum ulcer was also healing well.

This is the third published report of these two rare conditions occurring in one patient. In our patient the subcorneal pustular dermatosis preceded the onset of the pyoderma gangrenosum by 6 years, but, unfortunately, immunoglobulins were not measured initially and we are unable to say whether the paraprotein band was present all along. Both pyoderma gangrenosum and subcorneal pustular dermatosis have been reported separately in association with an IgA paraproteinaemia. There are no reports of these two skin diseases occurring together except in these three cases with a co-existent serum IgA band, suggesting that the IgA paraprotein provides the crucial link between them. There is no evidence as to the possible role of IgA in the pathogenesis of either disease and direct immunofluorescence has been negative in all three cases.

Both diseases are characterized by intense neutrophilic infiltration of the dermis and are similar in this respect to erythema elevatum diutinum (Dowd & Munro, 1983), another condition which has been reported in association with IgA paraproteinaemia. Further evidence is needed to clarify the spectrum of the neutrophilic dermatoses and the possible significance of IgA in relation to them.

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# *Book Reviews*

**Recent Advances in Dermatology, Number 7.** Edited by R.H.Champion (1986) Edinburgh: Churchill Livingstone. ISBN 0-443-03231-9. Price £24.00.

Of the tweleve topics selected for review in this volume, seven are accounts of areas in which there have been genuine recent advances. The remaining five read rather more like a literature review prior to embarking on a research project. It is to be hoped that the writers of these particular chapters have recognized the need for research in the various fields. The volume begins with an excellent chapter by Pye and Sikora on 'New developments in molecular biology and immunology'. Many clinically-minded dermatologists may be tempted to skip the first three chapters of this volume and move to the more familiar clinical browsing grounds. They should resist this temptation, pour a glass of appropriate fluid, and settle down with Chapter one. It is one of the best accounts I have read of current advances in genes, oncogenes, cell cloning, mediators of inflammation and monoclonal antibodies. It deserves a much wider readership than a purely dermatological one. Armed with the knowledge acquired in Chapter one, no individual would have problems in understanding or profiting from the book's excellent review on collagen genes and proteins in human disease in Chapter two.

The more clinically-orientated chapters include drug eruptions, specific desensitization, the teeth in dermatology, a traditional review of therapy, and a well-written chapter by Ridley on vulval disease. The author's teaching skill is well illustrated when she begins by both listing and referencing four fascinating and somewhat esoteric case reports, which she then tells us will not be discussed further, a sure way of implanting information in the memory.

There is also a chapter on dermatological surgery in which Moh's technique is clearly described, and the book ends with a section on alternative medicine and dermatology. I turned to this with interest to see if I could identify the compounds described to me by patients who alternate between conventional medicine and homeopathy, but found no firm information in this area.

This volume compares well with volumes in comparable review series which have recently appeared in competition to the 'Recent Advances' series. There are a number of minor irritating referencing inaccuracies, but overall the book is useful and good value for the stated price.

Rona M.Mackie

**Practical Psoriasis Therapy.** N.J.Lowe (1986) Chicago: Year Book Medical Publishers, Inc. Pp. 201. Price £38.00.

There is a need for a book on practical psoriasis therapy; this book 'is intended for a broader audience than just dermatologists' and for the wider readership of general practitioners and dermatology nurses, this book provides a good review. Dr Lowe and 11 other authors combine to cover virtually all aspects of psoriasis management in 19 well-classified chapters. However, I found the coverage strangely unsatisfying, with too much repetition (inevitable with a multi-author book) and too little in-depth discussion.

For a 'practical' therapy book to give genuinely useful advice, the drugs discussed must be those available locally. Dr Lowe is aware of this and is well qualified to attempt to make this primarily American book of relevance in Britain by often giving British equivalents. Unfortunately, this has not been uniformly successful, for example in Table 4:1 and Chapter 7. A particular weakness for the British audience is the poor coverage of the use of retinoids because of the delay in wide availability of etretinate in the U.S.A. The descriptions of the use of 13 *cis*-retinoic acid for psoriasis are largely irrelevant in Britain and confusing to the non-dermatologist.

The colour plates, some of which seem to have been prepared to use as lecture slides, add little to the text. The chapter on histopathology seemed misplaced in this book: there are good basic descriptions in other dermatology text books. One particularly helpful feature is the publication of patient instruction leaflets covering many aspects of psoriasis management. These can easily be adapted for local use.

This is a practical, useful book for trainees in dermatology, general practitioners and dermatology nurses. It should not be placed in every medical library, but should be chained in the staff room area of the dermatology department where it can be read and referred to by all who care practically for patients with psoriasis. This book is well laid out, of convenient size, printed on good quality paper with good binding and is aesthetically pleasing to handle. Buy one for your staff; do not expect much depth in it for yourself, but you will find yourself referring to it.

A.Y.FINLAY

**Fundamentals of Skin Biopsy.** JUNE K.ROBINSON (1986) Chicago: Year Book Publishers. ISBN 0-8151-7312-1. Pp. 124. Price £24.00.

This book is written by an associate professor of dermatology and surgery at an American university and the contents are aimed at the fledgling American dermatological surgeon. Nevertheless, this book does have some sound advice for the British dermatologist wishing to perform minor skin surgery principally for diagnostic or therapeutic reasons. We are performing more biopsies now—therefore, we must be able to obtain the best possible specimen along with the best cosmetic result. The ideal training is, of course, in the operating theatre with an experienced surgeon, but a good step-by-step manual would help those who cannot get 'hands on' experience.

There are 13 chapters which run sequentially through a biopsy. A salutary chapter on when not to biopsy precedes a useful chapter on the techniques of local anaesthesia; this includes a reasoned discussion on the pros and cons of added adrenalin. Throughout, the book emphasizes (a) that you must plan a biopsy, (b) where you should place the incision and (c) the question how do you propose to close it and with what? Even the most experienced surgeon will come across unforseen problems; thus, there is a clear explanation of how to deal with such problems as 'dog ears', wounds that will not close and haematoma formation.

The text of the book is clearly and logically presented, the line drawings are simple and to the point, and it is therefore such a pity that the black and white photographs that have been included are of such poor quality as to make the recognition of any detail impossible. The poor quality of these photographs is doubly disappointing because it is their inclusion presumably that has made the book so expensive.

S.I.WHITE

**Fungal Dimorphism, with Emphasis on Fungi Pathogenic for Humans.** Edited by P.J.Szaniszlo (1985) New York: Plenum Press. Pp. 413. US $65.00.

Changes of cell shape frequently correlate with the status of certain fungi as saprophytes or pathogens. This means that the study of morphogenesis in pathogenic fungi is relevant to appreciation of their clinical behaviour as well as to the more general understanding of processes of cellular development and differentiation. This book is a valuable compilation of knowledge in the field of fungal dimorphism. Each chapter is a review article written by a scientist with particular experience of an individual dimorphic fungal pathogen.

An introduction by the editor of the book, and a beautifully illustrated general chapter on electron microscopic aspects of fungal cytology by Garrison, are followed by chapters devoted to individual fungal species: *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Candida albicans*, *Exophiala weneckii*, *Wangiella dermatitidis*, *Chrysosporium parvum*, *Phialophora verrucosa*, *Coccidioides immitis* and *Mucor* species. All the reviews are excellent, but the chapter on *C. immitis*, by Cole and Sun, has to be singled out as truly stunning.

I had hoped that a book covering so many dimorphic fungi might reveal some general insights into mechanisms of morphological change. However, it is the enormous differences between the causes and processes of dimorphism in different species that emerge most strikingly, not the similarities. Cell wall structures inevitably play a key role in determining the shape of fungal cells, but across the different species there is no consensus view of particular cell wall structures or modes of synthesis that relate to morphogenetic development. Cyclic AMP is cited in many of the chapters as a mediator associated with morphogenesis, but this ubiquitous molecule is involved in so many aspects of metabolic regulation that it would be unreasonable to suppose its action is necessarily always involved directly in dimorphic change.

In an age when analytical genetic approaches are applied so widely to biological problems, relatively few dimorphic fungal pathogens have been subjected to genetic scrutiny. Nevertheless, in several chapters there is evidence that this situation is changing and that experimentaion with fungal DNA is beginning to provide valuable new insights into the processes of fungal dimorphism and their regulation.

F.C.ODDS

**Advances of Dermatology. Volume 1.** Edited by J.P.Callen (1986) Chicago: Year Book Medical Publishers, Inc. ISBN 0-8151-1412-5. Price £38.00.

This latest addition to the series of advances, recent developments, and other publications of that ilk is, we are told in the preface, Volume 1 in this series, but has arisen on the basis of two volumes entitled *Current Issues of Dermatology*, published by an alternative publishing house, but carrying the same editorial board.

Fourteen chapters are divided into headings under Internal Medicine, Paediatric Skin Disease, Dermatopathology, Dermatologic Surgery and Clinical Research. In the first section I found the chapter on lupus erythematosus subsets most useful, and in the second section the part-chapter on genital warts in childhood and concern about sexual abuse was certainly cause for thought. The two areas covered in dermatologic surgery are nail surgery and lipo-suction, and the areas covered in clinical research include the human papilloma virus, ETAF and immunoglobulin deposition in patients with lupus erythematosus.

A novel introduction in this particular volume is a comment section at the end of each chapter. I feel that this is an interesting idea which is well worth developing. In this first volume the most valuable comment sections are those where the commentators are not afraid to disagree, or take issue, with the chapter writer. This is obviously much more stimulating than a rather bland paragraph at the end of the chapter stating that it is so well written that nothing more can be said. I would recommend that the editors continue the comments in a future edition, but that they encourage those invited to write comments to introduce some element of controversy. This will make much more interesting reading.

RONA M.MACKIE

## Books received

**Sexually Transmitted Diseases; Guide to Diagnosis and Therapy.** R.C.NOBLE. 3rd edition (1985) New York: Medical Examination Publishing Co. Pp. 350. Price $ 33·50.

**Roxburgh's Common Skin Diseases.** J.D.KIRBY. 15th edition (1986) London: H.K.Lewis. Pp. 432. Price £16.00, hard cover, £10.00 soft cover.

**Essentials of Dermatology.** J.L.BURTON. 2nd edition (1985) Edinburgh: Churchill Livingstone. Pp. 160.

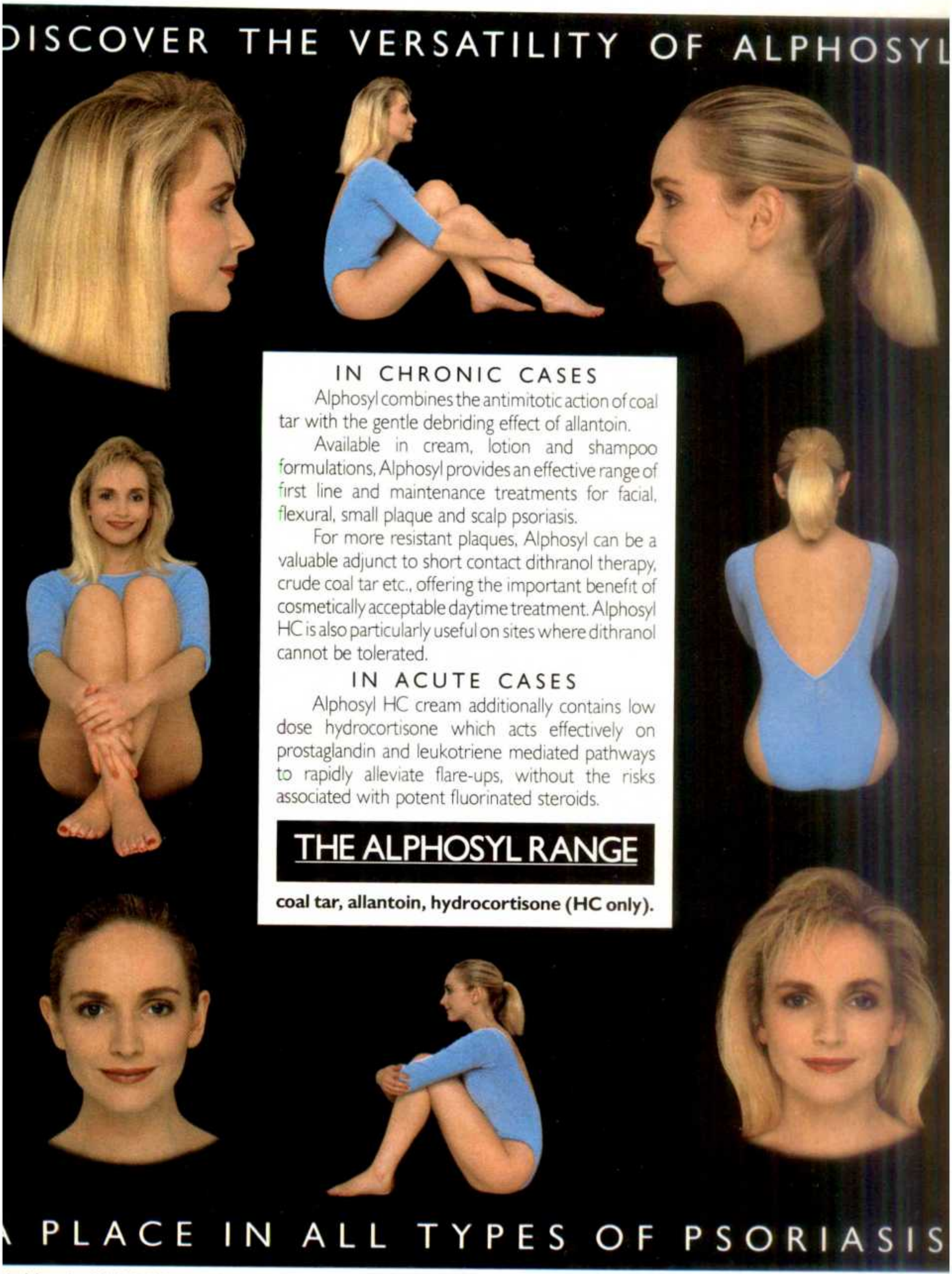
*British Journal of Dermatology* (1986) **115,** 122.

# *News and Notices*

## NATO Advanced Study Institute on Toxicology of Pesticides: Experimental, Clinical and Regulatory Perspectives

The above NATO Advanced Study Institute on Toxicology of Pesticides is to be held in Riva del Garda, Italy from 6 to 15 October 1986.

The directors are L.G.Costa (U.S.A.) and C.L.Galli (Italy). For further information please contact the Organizing Secretariat: Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23–20121 Milan, Italy. Telephone: (02) 702 267 and 783 868. Cable: Lorenzfound.



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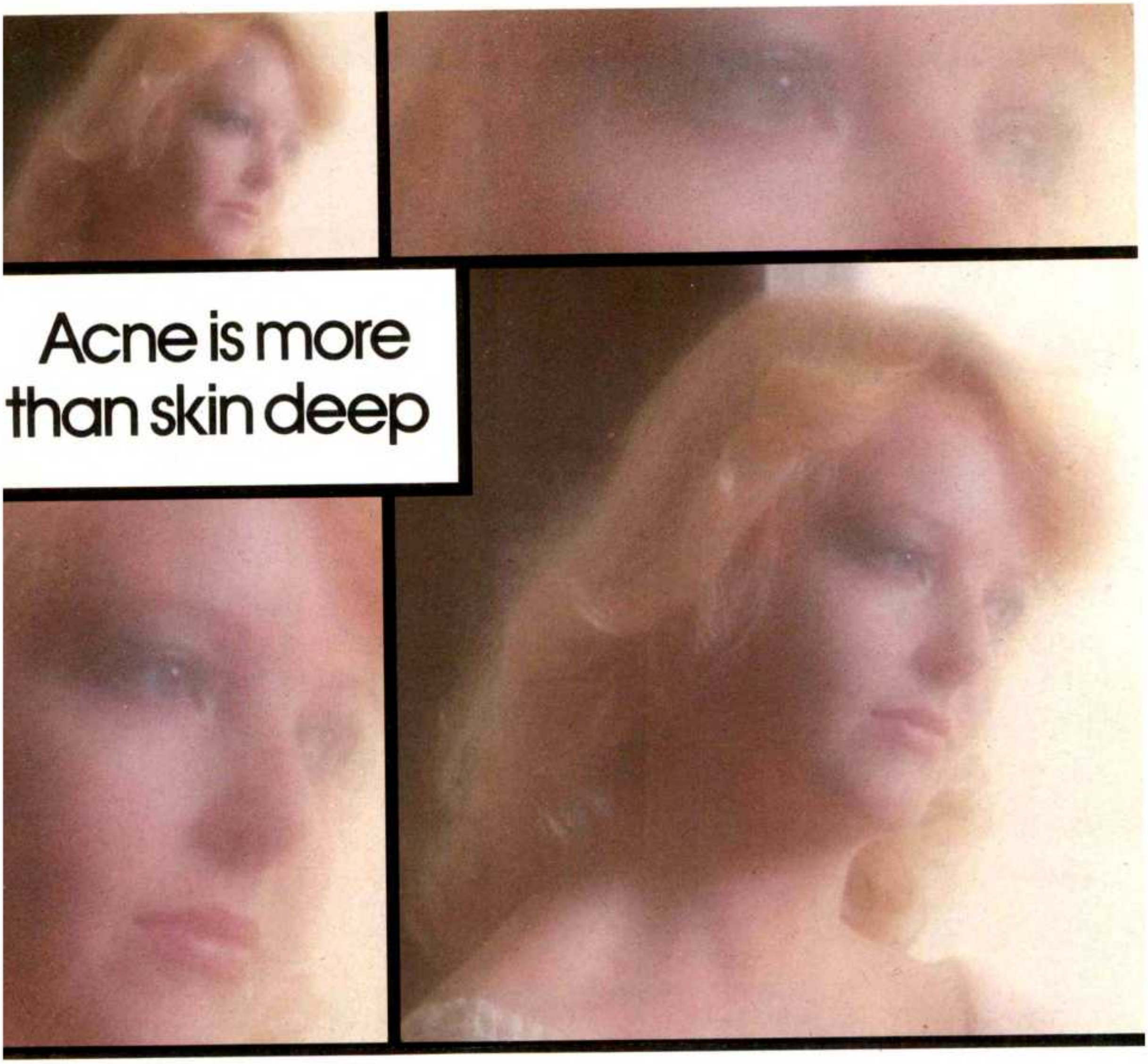
tion PC. Apply shampoo to wet hair, massage to lather and rinse. Repeat if necessary. **Contra-indications, warnings etc.** Sensitivity to coal tar. Discontinue if irritation occurs. External use only. **Presentati**
alcoholic extract of coal tar 5%, allantoin 2% (Application PC 0.2%). **Basic NHS Cost** Cream 75g, £1.56. Lotion 250ml, £2.27. Application PC 60g, £0.48. **Product Licence Nos.** Cream 0036/50
036/5008. Application PC 0036/5005. ALPHOSYL HC. **Uses** Treatment of inflamed psoriasis. **Dosage & Administration** Apply 2 to 4 times daily. **Contra-indications, warnings etc.** Should not be used in s
ns. Should not be used extensively in pregnancy. Avoid long-term use in infants. **Presentation** Contains refined alcoholic extract of coal tar 5%, allantoin 2%, hydrocortisone PhEur 0.5%. **Basic NHS cost** 3
5g, £2.41. **Product Licence No.** 0036/0026. Further information is available from Stafford-Miller Limited, Professional Relations Division, Hatfield, Herts. AL10 0NZ.



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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This Journal is covered by *Current Contents*, *ISI/BIOMED*, *Science Citation Index* and *ASCA*.

**Manuscripts.** Three copies of all material for publication should be sent to the Editor, Professor Rona M.MacKie, Dermatology Department, Glasgow University, Glasgow G12 8QQ. Manuscripts should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

**Abbreviations and units.** Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

| | | | | | |
|---|---|---|---|---|---|
| gram(s) | g | minute(s) | min | molar | mol/l |
| kilogram(s) | kg | centimetre(s) | cm | millilitre(s) | ml |
| milligram(s) ($10^{-3}$g) | mg | second(s) | s | gravitational acceleration | *g* |
| microgram(s) ($10^{-6}$g) | μg | cubic millimetre(s) | mm$^3$ | micrometre(s) | μm |
| nanogram(s) ($10^{-9}$g) | ng | millimetre(s) | mm | per cent | % |
| picogram(s) ($10^{-12}$g) | pg | millicurie(s) | mCi | isotopic mass number placed as | $^{131}$I |
| hours(s) | h | milliequivalent | mmol | | |

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations, A Guide for Biological and Medical Editors and Authors* (1977) The Royal Society of Medicine, London.

**Illustrations** should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be *unmounted* glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these should be grouped on a separate sheet of paper.

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**References.** Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be given in the Vancouver style, i.e. references appear as superscript numbers in the text, e.g. '. . . our previous report[1] and that of Smith *et al.*[2] . . .', and are brought together numerically in the reference list at the end of the article in order of their first mention in the text. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of journal in full; (5) volume; (6) number of the first page of the article. *Thus*: CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to books and monographs should include: (1) author(s) or editors(s); (2) year of publication; (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: BEARE, J.M. & WILSON JONES, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

**Page proofs** will be submitted without the original typescript to the author for proof-correction (see under 'manuscript') and should be returned to Blackwell Scientific Publications within three days. Major alterations from the text cannot be accepted.

# British Journal of Dermatology, Volume 115, Number 1, July 1986

CONTENTS PAGE

## Laboratory and Clinical Investigations

Vehicle effects on percutaneous absorption: *in vivo* and *in vitro* comparisons with human skin 1
R.L.BRONAUGH AND T.J.FRANZ

Monoclonal antibodies against epidermal cell surface and dermal-epidermal junction: antigens which may be epidermal cell spreading factors 13
H.KATAYAMA, J.KINO, S.ITAMI, M.TSUTSUI, H.KOIZUMI, K.M.HALPRIN AND K.ADACHI

Lichen planus in relation to occupational and non-occupational exposure to chemicals 23
C.LIDÉN

Nickel dermatitis: the reaction to oral nickel challenge 33
D.J.GAWKRODGER, S.W.COOK, G.S.FELL AND J.A.A.HUNTER

Electrical field stimulation of isolated primate sweat glands 39
G.I.VITALE, R.P.QUATRALE, P.J.GILES AND J.E.BIRNBAUM

Gianotti-Crosti syndrome: a study of 26 cases 49
A.TAÏEB, PATRICE PLANTIN, P.DU PASQUIER, G.GUILLET AND J.MALEVILLE

Incontinentia pigmenti: eosinophil chemotactic activity of the crusted scales in the vesiculobullous stage 61
H.TAKEMATSU, T.TERUI, W.TORINUKI AND H.TAGAMI

Use of UV-A sunbeds for cosmetic tanning 67
B.L.DIFFEY

## Pharmacology and Treatment

Treatment of polymorphous light eruption with nicotinamide: a pilot study 77
R.NEUMANN, E.RAPPOLD AND HELGA POHL-MARKL

The surgical treatment of upper limb hyperhidrosis 81
P.S.MALONE, A.E.P.CAMERON AND J.A.RENNIE

## Case Reports

Concurrent multiple morphea and neonatal lupus erythematosus in an infant boy born to a mother with SLE 85
N.OHTAKI, C.MIYAMOTO, M.ORITA, M.KOYA AND M.MATSUO

Giant solitary trichoepitheliomas located in the perianal area: a report of three cases 91
F.M.TATNALL AND E.WILSON JONES

Microcystic adnexal carcinoma 101
F.RONGIOLETTI, E.GROSSHANS AND A.REBORA

Atrophia maculosa varioliformis cutis 105
V.J.MARKS AND O.F.MILLER

Three cases of eccrine porocarcinoma 111
L.PUTTICK, P.INCE AND J.S.COMAISH

## Correspondence

Subcorneal pustular dermatosis followed by pyoderma gangrenosum 117
V.A.VENNING AND T.J.RYAN

**Book Reviews** 119

**News and Notices** 122

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# Foreword

This annual supplement contains details of the programme, and summaries of the papers, posters, and clinical cases to be presented at the 66th Meeting of the British Association of Dermatologists, to be held in Sheffield on 9–12 July 1986.

Thanks are due to Glaxo Laboratories for generous financial assistance, and to the Department of Medical Illustration, Royal Hallamshire Hospital, Sheffield, for production of clinical photographs.

Rona M.MacKie

DR R.E.CHURCH

*President of the British Association of Dermatologists*
1985–1986

The Royal Hallamshire Hospital, Sheffield

*British Journal of Dermatology* (1986) **115**, Supplement **30**, 5–9.

# British Association of Dermatologists Sixty-Sixth Annual Meeting, 1986, Sheffield

PRESIDENT DR R.E.CHURCH

## PROGRAMME OF MEETING

| | | |
|---|---|---|
| **Tuesday 8 July** | | |
| **Biology Lecture Theatre 1** | | |
| 09.15 | British Contact Dermatitis Group | Symposium |
| **Octagon Centre** | | |
| 14.00 | British Society for Dermatopathology | Symposium: Malignant melanoma and related problems |
| **Wednesday 9 July** | | |
| **Biology Lecture Theatre 1** | | |
| 09.00 | British Contact Dermatitis Group | Short papers |
| **Octagon Centre** | | |
| 09.00 | British Society for Dermatopathology | Symposium and slide seminar: Malignant melanoma and related problems |
| **Biology Lecture Theatre 2** | | |
| 09.00 | British Dermatological Surgery Group | Short papers |
| **Children's Hospital Lecture Theatre** | | |
| 09.00 | British Society for Paediatric Dermatology | Short papers |
| **Octagon Centre** | | |
| 13.00 | Registration desk opens | |
| 14.00 | B.A.D. Annual General Meeting | |
| **Thursday 10 July** | | |
| **Octagon Centre** | | |
| 08.45 | Registration desk opens | |
| | **Symposium: Hand Eczema** | |
| 09.00 | Dr Etain Cronin | Patterns of hand eczema |
| 09.20 | Professor S.Fregert | Consequences of positive patch test reactions in hand eczema |
| 09.40 | Dr Ingela Rystedt | Work related hand eczema in atopics |

| | | |
|---|---|---|
| 10.00 | Dr R.J.G.Rycroft | Allergic contact dermatitis can affect any part of the hand |
| 10.20 | Dr D.Burrows | Role of metals in hand eczema |
| 10.40 | **Coffee** | |
| | **Proffered Papers** | |
| 11.10 | R.A.Logan and W.A.D.Griffiths | Experience with etretinate for 'PUVA-dependent' psoriatics |
| 11.25 | B.E.Monk and S.M.Neill | The role of alcohol consumption in the natural history of psoriasis |
| 11.40 | D.M.Mitchell, R.J.Johnson, H.J.Testa, N.Y.Haboubi and R.J.G.Chalmers | Ultrasound and radio-isotope scans are poor indicators of liver damage in psoriatic patients treated with methotrexate |
| 11.55 | T.J.Phillips, P.Wallis and H.Baker | Pulmonary function in patients on long-term, low-dose methotrexate |
| 12.10 | N.H.Cox, D.J.Downey, E.J.Tuyp, S.K.Jones, J.L.Jay, H.Moseley and R.M.MacKie | Safety of photochemotherapy—an eight-year follow-up study |
| 12.25 | D.Chester Kalter | The epidemiology of *Trichosporon beigelii* infection in Houston, Texas |
| 12.40 | B.Adriaans, B. Draser and R.Hay | The role of anaerobic bacteria in the pathogenesis of tropical (phagedenic) ulcer |
| 12.55 | **Lunch** | |
| 14.00 | Professor C.E.Orfanos Guest Lecturer | Retinoids in dermatology |
| 14.20 | M.M.Walker, C.E.M.Griffiths, J.N.Leonard, A.V.Powles, J.Weber, W.Harris and R.J.Clayton | Dermatological conditions associated with HTLV–III virus infection |
| 14.35 | J.K.Rivers, P.D.Samman, M.F.Spittle and N.P.Smith | Pityriasis lichenoides-like lesions associated with poikiloderma: a precursor of mycosis fungoides |
| 14.50 | R.A.Logan, M.F.Spittle and N.P.Smith | Photochemotherapy for cutaneous T-cell lymphoma—the St John's experience |
| 15.05 | S.Whittaker, C.J.F.Spry and R.Russell Jones | Lymphomatoid papulosis: a rare manifestation of the hypereosinophilic syndrome |
| 15.20 | C.I.Harrington and I.R.Dunsmore | An investigation into the incidence of auto-immune disorders in patients with localized morphoea |

| | | |
|---|---|---|
| 15.35 | **Tea** | |
| 16.00 | V.A.Venning, P.A.Frith, A.J.Bron, P.R.Millard and F.Wojnarowska | Ocular and oral involvement in bullous and cicatricial pemphigoid: a clinical and immunopathological study |
| 16.15 | A.H.M.Heagerty, M.J.Tidman, I.M.Leigh, A.Jassim and R.A.J.Eady | Dystrophic epidermolysis bullosa: monoclonal antibodies reveal heterogeneity within clinical groups |
| 16.30 | **Viewing of Posters** | |

**Friday 11 July**
**Octagon Centre**

| | | |
|---|---|---|
| 08.45 | P.M.Dowd, I.C.Gaywood A.B.Kurtz and M.E.Shipley | Diabetic sclerodactyly |
| 09.00 | T.Phillips, I.M.Leigh, M.Hackett, A.Brain, P.Purkis and H.Navsaria | Allografts of cultured keratinocytes in clinical practice |
| 09.15 | J.S.Dover, A.Kobza Black and M.W.Greaves | A clinical and laboratory study of delayed pressure urticaria |
| 09.30 | A.W.Macfarlane, R.K.Curley and R.M.Graham | Recurrence rates of basal cell carcinomas according to site, method of removal, histological type and adequacy of excision |
| 09.45 | N.A.Swanson, C.B.Zachary and R.C.Grekin | Modes of spread of basal cell carcinoma |
| 10.00 | M.G.C. Dahl and A.J.Malcolm | Adapting Mohs' micrographic surgery to the needs and resources of the National Health Service |
| 10.15 | S.S.Bleehen, S.B.Bittiner and B.L.Priestley | Incontinentia pigmenti achromians of Ito. A neurocutaneous disorder |
| 10.30 | **Coffee** | |
| 11.00 | J.C.Roujeau, C.M.E.Rowland Payne, J.C.Guillaume, D.Penso, P.Saiag, J.Revuz and R.Touraine | Lyell's syndrome–Créteil's experience in 87 cases |
| 11.15 | S.E.Thomas and S.B.Bittiner | Prognostic factors in allergic contact dermatitis |
| 11.30 | J.S.C.English, I.R.White and R.J.G.Rycroft | Allergic contact dermatitis from 1-methylquinoxalinium-*p*-toluenesulphonate in a lithographic printing conditioner |

| | | |
|---|---|---|
| 11.45 | M.Kulozik, G.W.Cherry and T.J.Ryan | The importance of measuring the ankle/brachial systolic pressure ratio in the management of leg ulcers |
| 12.00 | R.Lever, D.Downey, K.Hadley, L.Baber, C.Hannigan and R.MacKie | A double-blind study of the effect of topical mupirocin in chronic atopic dermatitis |
| 12.15 | **Poster Discussion**<br>Three selected posters | |
| | R.A.Logan, B.Bhogal, A.K.Das, P.H.McKee and M.M.Black | Localization of bullous pemphigoid antibody—an indirect immunofluorescence study of 212 cases using a split-skin technique |
| | D.M.Mitchell, A.Smith, B.P.Rowan, T.W.Warnes, N.Y.Haboubi and R.J.G. Chalmers | The aminoterminal peptide of of type III procollagen as a serological marker of fibrosis in psoriatic patients treated with methotrexate |
| | W.F.G.Tucker, S.MacNeil, R.A.Dawson, S.Tomlinson and S.S.Bleehen | Increased calmodulin levels in psoriasis: cause or effect? |
| | W.F.G.Tucker, M.L.Wood, S.B.Bittiner, S.S.Bleehen and R.E.Church | Adverse risk factors in atopic eczema: clues from natal interpretation |
| | A.G.Messenger and A.E.Walker | Marie-Unna syndrome |
| | C.Maloney, M.L.Wood R.C.Spencer and C.I.Harrington | The cutaneous nodular chain: an unusual pattern of infection |
| | J.S.C.English, I.Foulds, I.R.White and R.J.G. Rycroft | Allergic contact dermatitis from an epoxy compound in a cutting oil |
| | C.B.Archer and D.M.MacDonald | Effects of topical and oral salbutamol therapy in atopic dermatitis |
| | R.J.Hay, Y.M.Clayton and G.Midgley | The use of itraconazole in chronic *Trichophyton rubrum* infections |
| | J.Ferguson, E.M.Walker and B.E.Johnson | Further clinical and investigative studies of chlorpromazine (CPZ) phototoxicity |
| | C.R.Lovell and G.Machin | Phototoxic plants in Britain |
| | S.Shaw, J.Wilkinson, M.Beck, C.Gobel and L.Chow | Micro-computer-based system for contact dermatitis |

| | |
|---|---|
| E.Young, M.Pepper, J.Everard, S.Shaw and J.D.Wilkinson | Attitudes to additives (a preliminary report) |
| S.Whittaker, N.P.Smith and R.Russell Jones | Solitary morphoea profunda |
| R.H.Meyrick Thomas, M.Rademaker, S.M.Grimes, I.B.Kovacs, A.MacKay, S.Bowcock, E.D.Cooke and J.D.T.Kirby | Oral nifedipine treatment for Raynaud's phenomenon secondary to systemic sclerosis—a double-blind, placebo-controlled study |
| M.J.Tidman and D.M. MacDonald | Cutaneous endometriosis: a histopathological study |
| J.N.W.N.Barker and D.M.MacDonald | Epidermal Class II HLA expression in atopic dermatitis |
| R.Cerio, M.H.Allen and D.M.MacDonald | The detection of extracellular antigens in formaldehyde-fixed paraffin-embedded cutaneous tissue |
| J.R.Gibson, S.G.Harvey, D.Kemmett and J.Salisbury | Treatment of common and plantar viral warts with human lymphoblastoid interferon-α |
| J.H.Barth, C.A.Cherry, R.P.R.Dawber and F.Wojnarowska | Clinical and laboratory manifestations of cutaneous virilism |
| M.Kulozik, S.Powell, T.J.Ryan and G.Cherry | Contact sensitivity in the community-based leg ulcer patients |
| D.A.Fenton, F.M.Pope, A.Temple, W.A.Galloway, G.Gasson, M.M.Black and J.D.Wilkinson | Ehlers-Danlos syndrome type VIII |

**Saturday 12 July**

09.15 **The Clinical Meeting will be held at the Royal Hallamshire Hospital, Glossop Road, Sheffield**

*British Journal of Dermatology* (1986) **115**, Supplement **30**, 11–28.

# SUMMARIES OF PAPERS

## Experience with etretinate for 'PUVA-dependent' psoriatics

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The combination of PUVA with the retinoid drug etretinate (Re-PUVA) has been demonstrated to have a PUVA-sparing effect in many centres (Lauharanta, Juvakoski & Lassus, 1981). Our experience at St John's using Re-PUVA in problem psoriatics is presented.

Twenty-three adult patients (13 males, 10 females, mean age 43 years) with severe psoriasis vulgaris were studied. Because of a lack of response to, or intolerance of, alternative anti-psoriatic treatments, these patients had required long-term maintenance PUVA [mean 4 years 2 months (range 11 months–6½ years); mean 210 PUVA exposures (72–408); mean 2097 J/cm$^2$ UVA (504–7577 J/cm$^2$)]. Etretinate was added either when psoriasis broke through maintenance therapy (17 patients) or when psoriais relapsed rapidly on discontinuing PUVA. The starting dose of etretinate was 50 mg/day (13 patients), others receiving doses ranging from 10–75 mg/day. Subsequent doses of etretinate and PUVA were adjusted on an individual basis.

Four patients cleared on etretinate alone. Re-PUVA cleared 11 completely, improved five considerably, and one further patient remained unchanged. However, PUVA doses were much reduced in the latter six patients. The remaining two patients had no benefit from Re-PUVA due to side-effects and non-compliance respectively. We were able to achieve a saving of 58% in UVA estimated dosage using Re-PUVA.

Side-effects of etretinate were well tolerated during a prolonged follow-up period (mean 17·6 months, range 7 months–2 years 4 months). One female patient was discovered to have vertebral hyperostoses after 18 months of etretinate therapy.

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## The role of alcohol consumption in the natural history of psoriasis

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The possible relationship of alcohol consumption to the course and severity of psoriasis has received little attention.

One hundred adult patients with chronic plaque psoriasis were studied. Based upon the history of their psoriasis over the preceding 5 years, they were divided into two groups—mild and severe. For the purpose of the study, severe psoriasis was defined as having necessitated the

TABLE 1. Medical and related problems due to alcohol in patients with psoriasis

Males

*Severe psoriasis* ($n = 9$)

- Korsakow's psychosis and possible alcoholic cardiomyopathy ($n = 1$)
- Alcoholic cirrhosis ($n = 2$)
- Severe non-cirrhotic alcoholic liver disease ($n = 2$)
- Alcoholism ($n = 1$)
- Head injury while crashing a stolen car when drunk ($n = 1$)
- Disqualified from driving for drink-drive offences ($n = 2$)

*Mild psoriasis* ($n = 1$)

- Alcoholic cirrhosis (cirrhosis anteceded onset of psoriasis) ($n = 1$)

Females

Severe psoriasis ($n = 1$)

- Alcoholic cirrhosis ($n = 1$)

patient's admission to hospital for treatment on at least one occasion, or requiring treatment with PUVA or cytotoxic drugs. Mild psoriasis was defined as having been controlled adequately on simple topical out-patient measures alone. Each patient's average daily alcohol consumption was assessed using the standard method described by Paton and Saunders (1981), and, based on this, each patient was designated either a heavy drinker or a temperate drinker. In addition, the medical case notes of each patient were reviewed for any history of possible alcohol-related medical problems.

The results show a striking difference between the sexes, in that only two of 45 women (4·4%) were heavy drinkers, compared with 14 of 55 men (25·5%). In male patients, only two of 23 with mild psoriasis (8·7%) were heavy drinkers, compared with 12 of 32 with severe psoriasis (37·5%). Alcohol-related medical problems were also significantly more frequently encountered in the men with severe psoriasis (Table 1).

Alcohol consumption in men with psoriasis was also compared with a group of men with chronic endogenous eczema; heavy drinking was found in only one of 27 patients (3.8%), and no relationship was found to the severity of the eczema.

Of the male patients with severe psoriasis admitted to hospital for treatment with the standard Ingram regime, there was no significant difference in the time taken to clear the psoriasis between the heavy and temperate drinkers, but heavy drinkers showed a significantly faster rate of relapse following discharge from hospital.

## REFERENCE

PATON, A. & SAUNDERS, J.B. (1981) ABC of alcohol: Definition. *British Medical Journal*, **283**, 1248.

## Ultrasound and radio-isotope scans are poor indicators of liver damage in psoriatic patients treated with methotrexate

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Two recent studies have claimed that ultrasound scans of the liver 'can be used to exclude significant hepatic damage' (Miller *et al.*, 1983) in patients receiving methotrexate for psoriasis, and that 'liver biopsies are not necessary when the ultrasound examination is normal' (Neild *et al.*, 1983).

In order to establish whether ultrasound scanning is indeed reliable for detecting significant liver damage, we studied 49 psoriatic patients who had been receiving methotrexate for at least one year (median 5·3 years; range 1·0–13·2 years). B scan and real time images of the liver were obtained on the day prior to liver biopsy, using a General Electron Datason DB scanner, by a radiologist (RJJ) or a qualified ultrasound radiographer. In view of earlier controversy regarding the value of radio-isotope scans, Technetium 99 sulphur colloid liver scans were performed on the same day and evaluated by an experienced reporter (HJT). All scans were reported without knowledge of the histology.

Neither method detected significant liver pathology, reliably, as revealed by biopsy. Ultrasound failed to detect patients with fibrosis or cirrhosis, and although colloid scans were slightly better in this regard, their specificity was poor. We have no reason to believe that scans performed in an average district general hospital would yield more information than we obtained, and we therefore recommend that neither method should be used as a substitute for liver biopsy.

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## Pulmonary function in patients on long-term, low-dose methotrexate

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Hepatotoxicity due to methotrexate is well documented, but pulmonary toxicity is less well recognized. We have recently encountered two patients who developed pulmonary complications after taking long-term, low-dose methotrexate for psoriasis. One patient developed an acute interstitial pneumonitis, with peripheral eosinophilia, which resolved after stopping methotrexate. The second patient developed an irreversible, progressive pulmonary fibrosis.

We therefore decided to investigate whether subclinical pulmonary toxicity could be detected in asymptomatic patients taking long-term, low-dose methotrexate for psoriasis. A group of 10 such patients was compared with 10 psoriatic patients who had never received any systemic treatment. Patients were matched closely for age, sex and smoking history. Respiratory symptoms were assessed using the MRC respiratory questionnaire, and physical examination, chest X-ray, electrocardiogram and full pulmonary function tests were performed in all patients.

We were unable to detect any changes indicative of early pulmonary damage; pulmonary function tests, including measurements of gas transfer, were within normal limits in every case. Results in the methotrexate group did not differ significantly from the control group.

Clinicians should consider methotrexate-pneumonitis in any patient taking the drug who develops respiratory symptoms in the absence of infection. Reassuringly, we have found no evidence of occult pulmonary damage in patients taking methotrexate on a long-term basis for psoriasis.

## Safety of photochemotherapy—an eight-year follow-up study

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Since 1977, 150 patients have been treated with PUVA in this department, mainly for psoriasis (74 patients) and mycosis fungoides (MF, 52 patients). Fifty-four psoriatics (73%) and 33 patients with MF (63%) were examined in 1985. Details of sun exposure, cutaneous carcinoma, actinic keratosis (AK) and PUVA freckles were recorded.

Six patients with MF had AK but none had carcinomas. Their mean age (69 years) was significantly greater than those without AK (58 years). Twenty patients with MF had PUVA freckles. Their mean cumulative UVA dose was 322 J/cm$^2$ compared with 265 J/cm$^2$ in those without freckles (difference not significant).

Two psoriatics had squamous carcinomas and one also had AK. Both had received methotrexate given concurrently with PUVA. Their cumulative UVA dose was 322 J/cm$^2$ and 1789 J/cm$^2$ (mean psoriatic dose 380 J/cm$^2$). Twenty-one psoriatics had PUVA freckles. Their mean cumulative UVA dose (564 J/cm$^2$) was significantly greater than those without freckles (264 J/cm$^2$).

Annual ophthalmological assessment was performed during and after PUVA therapy. Five patients (mean age 71 years) had lens opacities with significant visual impairment prior to PUVA therapy, and one had post-traumatic lens opacity and glaucoma, but developed no abnormality in the unaffected eye during 6 years follow-up (565 treatments). Three patients, mean age 61 years, developed new lens opacities during PUVA therapy (mean 157 treatments).

UVA transmission of the spectacles worn by patients currently attending for PUVA has been measured and will be presented.

## The epidemiology of *Trichosporon beigelii* infection in Houston, Texas

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*Trichosporon beigelii* infection is associated with the clinical disease of terminal hairs, white piedra. We investigated the prevalance of *T. beigelii* infection in 166 males and 36 females with genital complaints of any nature. Age, race, travel outside the U.S.A., symptoms, and clinical findings of infection were determined in each patient. Sixty-six (40%) males and five (14%) females had *T. beigelii* isolated by fungal culture of scrotal or labial hairs. Infection was more common among black patients ($P < 0{\cdot}001$), with 54% positive cultures, and a 6·4 times greater risk than in white patients by odds ratio analysis. Sixteen percent of white patients and 30% of all others had positive cultures. While 33% of 'travellers' were positive, 50% of those less mobile Americans were infected. Symptoms and clinical findings were found in 55% and 59% of culture positive patients, respectively. Two of the sexual contacts and primary family members cultured ($n = 13$) were positive for *T. beigelii.* Of the 30 specimens from non-genital sites in 15 patients with positive genital cultures, two axillary samples and one scalp sample were positive. Thirteen individuals became fungal culture negative spontaneously.

White piedra of the genital area has been reported only eight times in the U.S.A.; foreign travel has been implicated in many of these cases. No females have ever been clearly documented to have genital white piedra. Our results indicate that infection is common, is endemic in the U.S.A. and occurs in females. It is more common in black subjects, and may occasionally be transmitted to sexual partners. Clinical signs and symptoms vary from subclinical to marked in degree. A relationship may exist between *T. beigelii* and corynebacterium concurrently infecting genital hair shafts. Eradication of infection was extremely difficult, despite *in vitro* sensitivity to antifungal agents.

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## The role of anaerobic bacteria in the pathogenesis of tropical (phagedenic) ulcer

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Tropical ulcers are acute or chronic lesions characterized by dermal necrosis occurring in irregularly distributed endemic foci in the tropics. The pathogenesis remains obscure, although there is some evidence that it is transmissable (McAdam, 1966). Previous studies have reported fusiform bacteria and spirochaetes in smears (Lowenthal, 1963), but no attempts have been made to isolate these anaerobes in culture.

In this study, smears and aspirates have been taken from 120 patients with tropical ulcers in four endemic areas. Using pre-reduced tubes, organisms can be isolated after transportation from remote areas. Anaerobic bacteria were isolated predominantly from tropical ulcers of less than one month's duration and comprised Fusobacteria, *Bacteroides* and *Veillonella sp.* and anaerobic gram-positive cocci. Identifications were confirmed by biochemical methods and gas liquid chromatography. Anaerobes were always accompanied by facultative anaerobes or aerobic bacteria, suggesting a synergistic infection. Using six different cell lines, we showed that extracts of Fusobacteria are cytotoxic *in vitro*, affecting Vero and INT-407 cell lines predominantly. It is likely that a tropical ulcer develops in response to toxins originating from predominantly anaerobic bacteria which have entered into the dermis.

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MCADAM, I. (1966) Tropical phagedenic ulcers in Uganda. *Journal of the Royal College of Surgeons of Edinburgh*, **11**, 96.

## Dermatological conditions associated with HTLV-III virus infection

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Increasing numbers of homosexual men with the acquired immuno-deficiency syndrome (AIDS), persistent generalized lymphadenopathy (PGL) and AIDS anxiety presenting with dermatological conditions led to the need for a dermatology out-patient clinic especially for these referrals. One hundred and ten patients have been seen in a period of 14 months. In assessing the prevalence of dermatological conditions in these patients, only those with known HTLV-III antibody serology have been included. These totalled 83 patients in four categories: 21 patients fulfilling the CDC criteria for AIDS (A), 38 patients with PGL (B), 14 homosexual males with positive serology for HTLV-III antibody (C) and 10 homosexual males with negative serology for HTLV-III antibody (D).

Kaposi's sarcoma was the most common skin condition in AIDS patients, occurring in 12 (57%). Two patients presented with unusual skin manifestations of opportunistic infections—histoplasmosis and cryptococcosis. Vasculitis was noted in four AIDS patients. Seborrhoeic eczema was seen in four patients.

In the PGL group, 12 (31%) patients had seborrhoeic eczema of recent onset, truncal folliculitis was noted in 15 (39%), and oral hairy leucoplakia of the tongue occurred in three.

In the 24 patients in Groups C and D, seborrhoeic eczema was seen in six patients (42%) with positive serology, and in one patient with negative serology. A variety of other co-incidental skin conditions was seen in this group—patients having sought advice in the knowledge that skin lesions may be associated with AIDS.

## Pityriasis lichenoides-like lesions associated with poikiloderma: a precursor of mycosis fungoides

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The co-existence of pityriasis lichenoides and poikiloderma was reported by Samman (1971). Two of his seven patients developed mycosis fungoides.

Review of all patients from 1960 to 1986 at the St John's Hospital Skin Tumour Unit identified 13 cases of pityriasis lichenoides-like lesions in association with poikiloderma. Six of these 13 cases (46%) went on to develop mycosis fungoides. The duration of disease ranged from 2–34 years, with an average of 15·3 years. The pityriasis lichenoides-like lesions and patches of poikiloderma behaved independently of each other, often affecting different sites. Spontaneous regression was the rule with the pityriasis lichenoides-like lesions, but was exceptional in the poikilodermatous areas.

The histology of the pityriasis lichenoides-like lesions was either that of pityriasis lichenoides or lymphomatoid papulosis. The eventual development of mycosis fungoides was confirmed histologically in all six cases. One patient died with a poorly differentiated T-cell lymphoma, the remainder having plaque stage disease without evidence of systemic involvement.

The incidence of mycosis fungoides occurring in patients with poikiloderma is approximately 16% (Lambert, 1985), and although malignant lymphomas of various types have been reported in association with lymphomatoid papulosis, progression of lymphomatoid papulosis to mycosis fungoides has rarely been documented.

The association of pityriasis lichenoides/lymphomatoid papulosis and poikiloderma appears to be a significant risk factor for progression to mycosis fungoides.

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## Photochemotherapy for cutaneous T-cell lymphoma—the St John's experience

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Oral 8-methoxypsoralen photochemotherapy (PUVA) is an effective treatment for the early stages of cutaneous T-cell lymphoma (CTCL) (Bleehen, Vella Briffa & Warin, 1978; Warin, 1981). To date, 149 cases of CTCL have received, or are receiving, PUVA at St John's Hospital, London. Some earlier cases have now been followed up for 8 years since their first PUVA treatment.

Our present staging classification is based on clinical examination, examination of peripheral blood film and histological evaluation of skin, lymph nodes and viscera. The cutaneous lesions are staged thus: I macular disease; II plaque stage; III tumours, nodules or ulceration; IV erythroderma alone; V erythroderma with I, II or III.

Patients with macular disease are usually cleared by PUVA, and we have noted remissions of over 3 years following discontinuation of treatment. Despite PUVA, Stage II patients may progress to tumour stage disease. Most patients with tumour (Stage III) disease required therapy additional to PUVA. Erythrodermic cases (Stages IV and V) tolerated PUVA poorly.

Physical side-effects of PUVA are generally well tolerated by patients with early stages of CTCL, and irregular attendance has been the most common reason for an apparent poor clinical response to PUVA. The effect of photochemotherapy on the long-term prognosis of cutaneous T-cell lymphoma remains to be determined.

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## Lymphomatoid papulosis: a rare manifestation of the hypereosinophilic syndrome

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The hypereosinophilic syndrome (HES) is characterized by a persistent blood eosinophilia, evidence of internal organ involvement, and absence of parasitic or other known cause of eosinophilia. Cutaneous manifestations are generally non-specific, and consist of pruritic papules, urticaria or angio-oedema (Kazmierowski, 1978).

This study describes three adult male patients with HES who presented with a recurrent papulo-nodular eruption, and the histological features of lymphomatoid papulosis. Two patients also exhibited giant lesions which ulcerated and were diagnosed histologically as lymphomatous. However, the lesions were self-healing and immunohistochemical studies revealed a mature T-cell phenotype with a predominance of OKT4 positive cells. In addition, immunostaining with BE2, a monoclonal antibody which labels malignant T helper cells, was negative in both cases.

All three patients had endomyocardial fibrosis on biopsy, and a persistent eosinophilia (5–15 × $10^9$/l). Eosinophils were present in all cutaneous biopsy specimens, but in no case did they represent the predominant cell type. One patient developed paraplegia from an extradural leukaemic deposit. Chloracetate esterase-positive cells were present in the spinal lesion, but not in the cutaneous lesions.

The pathogenic relationship between HES and lymphomatoid papulosis remains speculative, but with two such rare disorders, it is unlikely to be coincidental.

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KAZMIEROWSKI, V.A., CHUSID, M.J., PARILLO, J.E., FAUCI, A.S. & WOLFF, S.M. (1978) Dermatologic manifestations of the hypereosinophilic syndrome. *Archives of Dermatology*, 114, 531.

## An investigation into the incidence of auto-immune disorders in patients with localized morphoea

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A study of patients with lichen sclerosus et atrophicus (LSA) has shown that they suffer from an increased incidence of other auto-immune disorders (Harrington & Dunsmore, 1981). The clinical and histological similarities of morphoea with LSA and the apparent co-existence of both disorders in some patients, prompted a similar study in patients suffering from localized morphoea.

Fifty females aged from 4 to 77 years (mean 36·8 years) with histologically proven morphoea were investigated. Age-matched controls were studied concurrently. A detailed personal and family history was taken for thyroid disease, alopecia areata, vitiligo, pernicious anaemia and diabetes. Each patient had blood examined for tissue antibodies.

Eight patients had an associated auto-immune disease compared with one control ($P < 0{\cdot}02$). Twenty-six patients had at least one positive auto-antibody compared with four controls ($P < 0{\cdot}0005$). Twenty patients had at least one relative suffering from an auto-immune disorder, but only five controls had affected relatives ($P < 0{\cdot}001$). The incidence of other disorders and affected relatives showed no significant relationship to the age of onset, duration, site, or number of lesions of morphoea, but positive antibodies were significantly more common in patients with more than one lesion ($P < 0{\cdot}025$).

As in LSA, patients with morphoea have an increased incidence of auto-immune disorders and tissue antibodies, and their families are more likely to have auto-immune disorders. These findings suggest that patients with morphoea should be followed up to detect the existence of other auto-immune diseases.

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## Ocular and oral involvement in bullous and cicatricial pemphigoid: a clinical and immunopathological study

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The relationship between bullous pemphigoid (BP) and cicatricial pemphigoid (CP) is uncertain. To investigate this relationship, 48 patients with BP and 10 patients with CP were evaluated clinically for mucous membrane involvement, and by direct immunofluorescence (IMF) of uninvolved oral mucosa and conjuctiva.

In BP patients, oral lesions occurred in 38% and eye symptoms in 50%; in CP the frequencies were 100% and 60%, respectively. Preliminary results from ophthalmological assessment show that ocular signs, including scarring, may occur in patients diagnosed as having BP, and with only minimal eye symptoms.

Linear deposits of IgG and/or $C_3$ in the basement membrane zone of uninvolved lip mucosa were seen in 13 of 23 BP patients, (including 6 of 11 whose disease was confined to the skin) and in 6 of 10 CP patients. Positive direct IMF at the basement membrane zone of conjunctival mucosa occurred in all of the three BP patients tested, including a patient with no symptoms referrable to the eyes. Two of five CP patients had positive conjunctival IMF.

The clinical and immunological ocular findings in BP have not been reported previously. Involvement of the ocular and oral mucosae in BP provides support for the concept that BP and CP are not distinct entities, but show considerable clinical and immunopathological overlap. Final classification of these disorders must await identification of the relevant antigen(s).

## Dystrophic epidermolysis bullosa: monoclonal antibodies reveal heterogeneity within clinical groups

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Because of the clinical similarity, it is often impossible to distinguish dominant dystrophic epidermolysis bullosa (DDEB) from the milder or localized form of recessive dystrophic EB (LRDEB). Accurate diagnosis is important, especially for genetic counselling. Electron microscopy (TEM) is unable to differentiate between DDEB and LRDEB by means of anchoring fibril analysis (Tidman & Eady, 1985).

The monoclonal antibodies LH 7:2 and LH 20 Sp were found to label normal epidermal basement membrane intensely as assessed by indirect immunofluorescence (IIF). Staining was also strong in cases of junctional EB ($n = 6$), EB simplex ($n = 2$) and DDEB ($n = 3$). In skin from patients with LRDEB ($n = 3$), staining was patchily reduced with LH 7:2, but generally weaker with LH 20 Sp. Staining with both monoclonal antibodies was absent in six cases of severe RDEB (SRDEB). In a further three patients, clinically diagnosed as having SRDEB, IIF staining was patchily reduced in two, and intense in the third. The diagnosis of these patients was subsequently modified.

We feel that these studies underline the existence of heterogeneity among DEB groups and that the new monoclonal antibodies now offer the best available method for distinguishing clinically confusing variants of DEB.

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## Diabetic sclerodactyly

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It has been reported recently that contractures of the finger joints occur in approximately 30% of juvenile onset insulin-dependent diabetes mellitus. One third of these patients also had thick, tight, waxy skin, most apparent over the dorsum of the hand. The presence of the lesions could not definitely be related to race, sex, dose of insulin or estimated control of diabetes, but was associated with an increased risk of subsequent microangiopathy. Biopsies of the skin only appear to have been performed in five patients, in which histology revealed an increase in the connective tissue in the lower dermis and death of glands in hair follicles. Decreased extractability of collagen suggesting increased cross-linkage and increased non-enzymatic glycosylation occurred in three individuals. It was suggested that glycosylation might alter cross-linkage of collagen and thus lead to waxy skin.

We now report the light and electron microscopic findings in 29 insulin-dependent diabetics (5 male and 2 female), mean age 45·3 years (range 26–67 years) with a mean duration of diabetes of 31·7 years (range 25–49 years), who have contractures of the finger joints and sclerodactyly most prominent clinically in the skin distal to the PIP joints. The associated complications of diabetes in these patients were retinopathy in 25 of 29, peripheral neuropathy in 24 of 29, autonomic neuropathy in of 4 of 7, and nephropathy in 7 of 29.

Biopsies for light and electron microscopic examination were taken from the dorsa of the fingers. Histology of the skin typically revealed acanthosis, hyperkeratosis, thickening of the dermis, dense dermal collagen and fragmentation and dumping of elastin. Small blood vessels showed ligative thickening of their walls and cutaneous nerves appeared degenerate. Electron microscopic findings revealed changes most prominent in elastin. There was a paucity of elastic fibres with dense septa composed of a microfibrillar substance between them. These microfibrillar dense zones (MDZ) were also present at the periphery of the fibre. Cystic spaces sometimes filled with fibre, fibrillar/granular material, occurred in some compact mature fibres. In other fibres the elastic matrix around the MDZ was completely granular with a disrupted moth-eaten periphery. Some fibres were transformed into irregular fibrillogranular bodies with a few dense regions at the periphery.

The nature of the cutaneous collagen in these patients, together with its degree of glycosylation and proteoglycan synthesis in both biopsies from both sclerotic skin and appropriate sections from normal skin, have also been investigated. The results of these studies will also be presented.

## Allografts of cultured keratinocytes in clinical practice

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Technical advances now permit the expansion of a given population of epidermal keratinocytes, so that a small sample of epidermis can be cultured to produce stratified sheets of epidermal cells. The clinical applications of this technique were first demonstrated in 1981, when autografts of cultured keratinocytes were applied to young burns victims (O'Connor *et al.*, 1981). Two years later, culture allografts grown from cadaver skin were successfully grafted onto partial thickness burns (Hefton *et al.*, 1983).

We have now used culture allografts in a wide variety of wounds, including chronic venous ulceration, rheumatoid ulcers, unhealed donor sites, and in plastic surgery patients undergoing removal of tattoos, keloid scars and rhinophyma. In the leg ulcer group, (patients with end stage venous ulcers unresponsive to conventional treatment), 50% of the grafts were successful. However, even in patients whose grafts failed to take, there was rapid pain relief and dramatic healing from the ulcer edge. This effect was monitored by regular measurements of the ulcer area.

Culture allografts provide a useful alternative to split skin grafting with good cosmetic results and avoidance of a painful donor site. They are useful biological dressings and may release a growth factor which appears to stimulate rapid epithelialization from the wound edge.

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## A clinical and laboratory study of delayed pressure urticaria

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Delayed pressure urticaria (DPU) is characterized by the episodic development of painful deep swellings, several hours after pressure application. Forty-four patients (24 males and 20 females) seen at St John's Skin Hospital since 1977, in whom DPU was reproducible with a graded pressure stimulus, were studied.

The mean age at onset of the disease was 33 years and the mean duration was 10 years. The mean time to onset of swelling after pressure application was $3\frac{1}{2}$ h, the mean peak at 10 h and the mean duration was 36 h. Areas most commonly affected were the hands, feet and extremities. Lesions were most often induced by standing or walking for long periods, or carrying a heavy object. One or more generalized symptoms, (malaise, fatigue, fever and/or headache) occurred at some stage in 29 patients (66%). The severity of DPU was enough to affect daily activities in 37 patients (85%).

More than 50% (16 of 29) of those tested also demonstrated delayed dermographism. Chronic urticaria occurred in 62% (27 of 43); 32% (14 of 44) had associated spontaneous angioedema and 14% (6 of 42) had acute dermographism. Positive laboratory findings were leucocytosis (without absolute eosinophilia) in 32% (14 of 44), immune complexes in 52% (15 of 29) and thyroid antibodies in 33% (8 of 24). There was no evidence of a specific decrease in plasma levels of protease inhibitors in any of 23 patients tested.

Treatment with antihistamines was generally ineffective. Eleven of 14 patients treated with systemic steroids improved, but only three had good control on less than 30 mg prednisone per day. However, side-effects prevented their long-term use. A double-blind controlled trial of indomethacin versus placebo in 14 patients did not show benefit in the dose regime used.

## Recurrence rates of basal cell carcinomas according to site, method of removal, histological type and adequacy of excision

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We have reviewed the treatment, histological type, completeness of excision and recurrence rate of 157 basal cell carcinomas (BCC) in 115 patients first seen between 1976 and 1982. All patients were examined for evidence of recurrence or the development of further BCCs, and the sections were evaluated for clearance of tumour and histological features.

The age range at presentation was 37–85 years (mean age 63·7 years). One hundred and thirty-four tumours had arisen *de novo* (primary BCCs), and 23 were recurrences following previous treatment. Of the 134 primary BCCs, 30 recurred (22·4%), and of the 23 previously treated BCCs, 10 recurred (43·5%). There was a significantly higher recurrence rate for those lesions treated by curettage and cautery compared to excision. Of tumours of the head and neck, the highest overall recurrence rate was for lesions on the temple and the lowest for lesions on the cheek. Evaluation of clearance by single mid-specimen section did not appear a reliable index of likely recurrence: excised lesions judged to be incompletely excised recurred no more frequently than those judged to be completely excised. With regard to histological type, those tumours classified as morphoeic recurred most frequently and nodulocystic lesions least frequently. A significant proportion of recurrences were only discovered when patients were recalled for examination.

## Modes of spread of basal cell carcinoma

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Basal cell carcinoma (BCC) is the most common form of skin cancer. In general, this is an indolent tumour with well defined local invasion and minimal risk of metastasis. However, certain presentations of this tumour can appear with a more aggressive growth pattern, as evidenced by more extensive and rapid local invasion. Frequently, such invasion is not clinically apparent due to microscopic extension along deep anatomic structures.

Six years of experience utilizing Mohs'micrographic surgery on 4000 tumours has allowed us to identify specific patterns of tumour spread. These may include spread along embryonic fusion and fascial planes, cartilage, tarsal plate and external root sheath of hair follicles, and may also be perineural and periosteal. This paper will describe the characteristic modes of spread of BCC, and will illustrate anatomic sites where more extensive tumour growth can occur, requiring more aggressive surgery, including microscopic control of margins.

## Adapting Mohs' micrographic surgery to the needs and resources of the National Health Service

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Mohs' surgery gives the highest cure rates of any treatment for basal cell carcinoma. It is indicated particularly for recurrent, ill-defined or extensive infiltrating lesions. The current method, employing frozen sections of fresh tissue, requires skilled histological technical assistance which makes it expensive. Moreover, the usual practice of multi-stage procedures in one day requires an open-ended commitment of operating time and personnel. Not surprisingly, the development of Mohs' surgery in the U.K. has been very limited.

We have developed techniques of tissue-handling which overcome most of these problems. After the standard Mohs' surgical excision and mapping, the tissue specimens are orientated on filter paper and flattened and immobilized in tissue-processing cassettes by compression with foam sponge. After fixation in formalin at 37°C for 4 h, the specimens, still immobilized in the cassettes, are dehydrated and processed to wax overnight, together with other routine specimens using automated equipment. Stained sections are available by midday the following day and, if necessary, the patient is recalled for further excision the next morning. Up to three excisional stages may be performed during the working week on Monday, Wednesday and Friday mornings, and the open-ended commitment of operating time is avoided. Consequently, micrographic surgery and other work can be planned more easily in advance. In 11 months, we have treated 47 lesions in 42 patients by this method. The median number of excisional stages required for margin clearance was two (range 1–7) and in 41 of the 47 lesions (87%), clearance was achieved in three stages or less, i.e. within 5 days. This method requires no special technical assistance and uses facilities which are already available in many N.H.S. hospitals. It should enable micrographic surgery to be developed more easily in the U.K.

## Incontinentia pigmenti achromians of Ito. A neurocutaneous disorder

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Five children (four male and one female) with the condition of incontinentia pigmenti achromians of Ito have been studied. All showed the characteristic 'marble cake' pigmentary changes with irregularly shaped, whorled and linear streaks of hypomelanosis on the trunk and limbs. Only one of the children was an Asian. The pigmentary changes were noted in early infancy and in none of the patients were there vesicobullous lesions. All showed some delay in motor development, and in three this was associated with hypotonia, presenting as floppy babies. One is profoundly mentally retarded and has epilepsy and megalencephaly. One child is clumsy with difficulty in co-ordination and mild developmental delay. Two others were slow in their development in infancy, but improved subsequently. Visual, hearing and skeletal abnormalities were also associated.

Light and electron microscopic studies were carried out on skin biopsies from four of these children and showed melanocytes to be present in normal numbers in the basal layer of the epidermis. In the hypomelanotic areas there was a lack of melanosomal complexes in the epidermal keratinocytes. A few dyskeratotic cells were seen in one of these biopsies. No significant pigmentary incontinence was noted.

Congenital anomalies, particularly of the central nervous system, are seen in incontinentia pigmenti achromians, which should be regarded as one of the congenital neurocutaneous disorders.

## Lyell's syndrome—Créteil's experience in 87 cases

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Eighty-seven patients (55 females and 32 males) with toxic epidermal necrolysis (Lyell's syndrome) were treated in a supra-regional dermatological intensive care unit at Créteil between 1972 and 1985. Ages ranged from 2–90 years (median 47 years). Epidermal necrosis affected 10–100% of body surface area (median 30%), and mucous membranes were almost always involved. The medicament highly likely to have been responsible was identified in 61 cases, most frequently sulphonamides, and butazone and oxicam-containing, non-steroidal anti-inflammatory drugs. Typically, the eruption began 7–21 days after first drug ingestion (median 15 days) and sooner on subsequent exposure. Systemic involvement was common, lymphopenia occurred in 88%, especially affecting T-helper cells, proteinuria in 61%, elevated transaminase levels in 56% and thrombocytopenia in 15%.

In the 22 patients who died (25%), ages ranged from 22–90 years (median 64 years) and epidermal necrosis was more extensive (median 50% of body surface area). Neutropenia and uraemia indicated a grave prognosis. Sepsis was the commonest cause of death, usually due to *Staphylococcus aureus* or *Pseudomonas aeruginosa*.

In survivors, late complications included a sicca syndrome in up to 40%, and blindness in one patient.

Treatment included nursing in an air-fluid bed, cutaneous antisepsis, local mouth and eye care, nasogastric feeding and fluid, and colloid and electrolyte balance. Systemic antibiotics were used as indicated, but steroids were not, as they are probably harmful.

## Prognostic factors in allergic contact dermatitis

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Two hundred and twenty patients, consisting of 92 atopics (A) and 128 non-atopics (NA), who yielded positive results on patch testing during the period 1975–80, completed a detailed questionnaire during 1985. 69 A (75%) and 59 NA (46%) continued to have dermatitis, a significant difference ($P < 0{\cdot}01$; chi squared test).

Hand dermatitis affected 135 patients, foot dermatitis 62 patients, facial dermatitis 63 patients and trunk dermatitis 78 patients. The dermatitis had persisted in 92, 40, 40 and 56 patients respectively. There was a total of 131 patients with a single site affected, of whom 67 (51%) did not improve. Eighty-four had multiple sites affected, of whom 62 (74%) did not improve. Those with multiple sites affected had a worse prognosis ($P < 0{\cdot}001$).

One hundred and thirty-nine patients were sensitive to a single allergen, and 64 of these improved. Twenty-six patients were sensitive to multiple allergens, and four of these improved. Patients with multiple allergens had a significantly worse prognosis ($P < 0{\cdot}001$). One hundred and nineteen patients had avoided their allergen, and 74 (62%) were improved. Ninety-one patients were unable to avoid their allergen, and 15 (16%) had improved. The difference between these groups was significant ($P < 0{\cdot}01$).

We compared 79 patients in high risk occupations with 51 patients who had changed to low risk occupations following patch testing. Dermatitis persisted in 49 (62%) of the high risk group compared with 27 (53%) of those who had changed jobs ($P < 0{\cdot}01$).

In conclusion, patients with allergic dermatitis have a worse prognosis when associated with atopy, multiple body sites, multiple sensitizers, inability to avoid the sensitizer, and a high risk occupation.

## Allergic contact dermatitis from 1-methylquinoxalinium-*p*-toluenesulphonate in a lithographic printing conditioner

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In 1983, dinitrophenyl pyridinium chloride (DNPC), the allergen in Instafax etch (Kodak), was replaced with 1-methylquinoxalinium-*p*-tolunesulphonate (MQTS), and the name was changed to PMT II conditioner. The conditioner contains MQTS at between 1% and 2%. It is applied by hand to the offset lithography plate to render the image receptive to ink. A swab held mainly between the thumb, index and middle fingers is often used for its application.

In 1985, four lithographic printers, currently using the PMT II system, presented with endogenous-looking finger-tip eczema, which resembled the DNPC allergic contact dermatitis and has proved difficult to clear. Positive patch test reactions were demonstrated to MQTS at 1% in $H_2O$ in all four printers, and progressively weaker reactions to 0·1% and 0·01% dilutions of MQTS in three printers.

It is recommended that the conditioner be applied once rather than several times for each print run, with a paint pad rather than with a swab, and while wearing nitrile or disposable plastic gloves.

## The importance of measuring the ankle/brachial systolic pressure ratio in the management of leg ulcers

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A recent community study on leg ulcers using non-invasive techniques to assess the blood supply found that venous valvular incompetence was the major aetiological factor, although a number of patients had arterial and mixed pathology.

The aim of the present study was to evaluate the extent of peripheral arterial disease using the resting ankle/brachial systolic pressure ratio measured with a portable Doppler ultrasound probe, and to determine the importance of this measurement in the healing rate of leg ulcers.

Seventy-six patients (144 limbs) with 93 leg ulcers were examined. Patients were being seen in the community and out-patient ulcer clinics. Healed patients were those in which the ulcers had been healed for at least one year.

Nineteen patients were found to have an abnormal resting ankle/brachial pressure ratio ($< 0{\cdot}9$). In 10 of these patients an abnormal ratio was found in both limbs, and in the other nine patients it was unilateral. Seven of the 19 patients had previously been classified as having venous ulcers. Sixty-one of the 76 patients were followed up with regard to healing. Seventeen patients (28%) were completely healed, and of these two (12%) had arterial insufficiency, 12 (70%) were of venous origin and three (18%) had a mixed aetiology. Thirty patients were classified as 'non-healers' (ulcers present for more than one year). In this group, three (10%) had arterial insufficiency, 17 (57%) had venous incompetency and 10 (33%) had arterial as well as rheumatoid arthritis and diabetes mellitus. Fourteen patients were healing based on serial photographic and planimetry measurements, and 93% of these patients' ulcers were due to venous incompetency. Fourteen patients were found to have arterial hypertension and were referred to their physicians for management.

Although arterial insufficiency is often diagnosed by clinical symptoms and physical examination, we have found that in a number of patients palpation of foot pulses is difficult, particularly in cases where excessive oedema is present. The fact that 57% of our patients who were non-healers had ulcers that were predominantly venous, would indicate that poor compression bandage was being applied, or other factors besides vascular causes could be impeding healing.

This study demonstrates the importance of assessing the blood supply of the limbs of patients with leg ulcers.

## A double-blind study of the effect of topical mupirocin in chronic atopic dermatitis

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Thirty-four patients satisfying the Rajka/Hanifin criteria for the diagnosis of chronic atopic dermatitis entered a double-blind study to compare the effect of topical mupirocin (Group A) or polyethyleneglycol base ointment (Group B), in addition to clobetasone butyrate and emollients. Sixteen patients in Group A and 14 in Group B were evaluable. Patients were assessed objectively on surface area of involvement, quantity of topical steroid required, and an objective scoring system using six indices of disease severity at eight body sites. Subjective assessment was based on the patient's view of changes in skin appearance, itch, and sleep disturbance. Statistical analysis was using *t*-tests for quantitative, and McNemar's test for qualitative, variables.

After 2 weeks topical application of mupirocin, Group A showed a highly significant improvement in appearance, in surface area involved, in severity score, itch, and sleep disturbance. This improvement was statistically significant both when Group A subjects were

compared with their own pre-mupirocin assessment scores, and when Group A subjects were compared with Group B subjects receiving base ointment. Semi-quantitative bacteriological assessment carried out throughout the study showed pre-treatment staphylococcus counts to be high and comparable in both groups. The mupirocin-treated group showed a greater reduction in staph. counts than the base-treated group.

These results confirm reports of staphylococcal colonization of the skin of atopic patients, and suggest that measures to reduce this colonization may be accompanied by clinical improvement.

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# SUMMARIES OF POSTERS

## Localization of bullous pemphigoid antibody—an indirect immunofluorescence study of 212 cases using a split-skin technique

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Epidermolysis bullosa acquisita (EBA) may initially present in a manner clinically indistinguishable from bullous pemphigoid (BP) (Gammon *et al.*, 1984a), before characteristic features of EBA develop some months later. Patients with EBA may prove resistant to therapy.

At present, routine immunofluorescence (IF) techniques do not distinguish between EBA and BP. EBA antigens are immunologically distinct, being localized below the lamina densa, whereas BP antigen is found both in the lamina lucida and basal keratinocytes. Using a 1·0 M sodium chloride split-skin technique (Gammon *et al.*, 1984b), we have re-examined the site of deposition of IgG anti-basement membrane zone (BMZ) antibodies in 212 sera from 'bullous pemphigoid' cases. These sera were sent for indirect IF techniques by the referring dermatologists, all with a suspected clinical diagnosis of BP.

The majority (94%) demonstrated linear BMZ fluorescence in the roof of the split, eight cases (3·8%) showed fluorescence in the base only and three cases (1·4%) showed a mixed pattern. The frequency of basal fluorescence found in this study was less than that detected by Gammon *et al.* (1984a), who found this pattern in 11 (13%) of 85 cases.

The sodium chloride split-skin technique is relatively simple and provides additional information on the antibody-binding site in cases of BP. This may allow a small minority to be identified as EBA.

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## The aminoterminal peptide of type III procollagen as a serological marker of fibrosis in psoriatic patients treated with methotrexate

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A simple test to monitor the development of fibrosis in patients requiring long-term methotrexate would be welcomed by dermatologists. Type III collagen, which predominates in developing hepatic fibrosis, is formed from type III procollagen by cleavage of peptide fragments which are released into the circulation in proportion to the amount of collagen laid down. Recently, a radioimmunoassay of the aminoterminal peptide of type III procollagen (PIIIP) has become available in commercial kit form (Behringwerke AG) and has been claimed to be of value in monitoring disease progression in fibrotic liver diseases (Hahn, 1984).

In a prospective study, we measured serum PIIIP concentrations at the time of liver biopsy in 51 patients who had received methotrexate for between 1·0 and 13·2 years (median 5·2 years). Lowest levels were found in those with normal histology and highest levels in those with cirrhosis. As a control, serum PIIIP levels are being measured in psoriatic patients not receiving cytotoxic therapy.

Serum PIIIP levels were significantly higher in patients with inflammatory and/or fibrotic liver histology than in those with normal findings or steatosis alone ($P = 0{\cdot}042$; Mann-Whitney U test). Isolated PIIIP measurements were insufficiently specific to discriminate between individuals with and without significant liver pathology. Sequential PIIIP assay may, however, be helpful in predicting liver damage in patients receiving methotrexate.

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## Increased calmodulin levels in psoriasis: cause or effect?

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We have shown that levels of calmodulin (CaM), the intracellular calcium-dependent regulator protein, are increased 2–3 fold in psoriatic lesions compared to controls (MacNeil *et al.*, 1985). CaM levels in uninvolved psoriatic epidermis are intermediate between control and plaque values.

We have therefore investigated the relationship between CaM levels and cellular proliferation by examining, firstly, other naturally proliferative skin conditions, i.e. a number of benign and malignant epidermal tumours, and secondly, by inducing proliferation artificially in both epidermis and peripheral blood lymphocytes.

Circulating lymphocytes were obtained from seven normal volunteers by standard Ficoll-Hypaque centrifugation methods, and incubated subsequently for 72 h with an optimally stimulating concentration of phytohaemagglutinin. Sellotape stripping was performed on the forearm skin of six normal volunteers, with epidermal shave samples being taken before and 24 h after the procedure. CaM was measured in all samples by biological activity assay, employing a CaM dependent phosphodiesterase enzyme.

The CaM levels obtained for the tumours varied between 1·05 μg/mg protein (basal cell carcinomas) and 1·89 μg/mg protein (viral warts), and were of a similar order to those found in uninvolved psoriatic epidermis (1·42 μg/mg protein), but considerably lower than levels measured in psoriatic plaque (2·29 μg/mg protein).

No significant increase in CaM content was observed after either mitogen stimulation of lymphocyte proliferation or Sellotape stripping of the epidermis.

The increased CaM content of psoriatic epidermis does not therefore appear to be directly related to its hyperproliferative state. Further work is needed to determine whether this alteration in CaM activity plays a causative role in the development of the psoriatic process.

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## Adverse risk factors in atopic eczema: clues from natal interpretation

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Several factors predisposing to the development of atopic eczema have been identified, foremost among these being a parental history of atopic disease (Fergusson, Horwood & Shannon, 1982). Infant feeding practices have been reported to influence the development of eczema, with early solid feeding and 'bottle' feeding particularly implicated (Matthew *et al.*, 1977). Other studies have failed to confirm any protective element in strict breast feeding in potentially atopic individuals (Mahood *et al.*, 1982), emphasizing the need for closer identification of the 'at risk' group.

We have reviewed the birth dates of 237 children with atopic eczema (128 boys, 109 girls, mean age 6·3 years) referred over a 2 year period. The 244 patients (120 boys, 124 girls, mean age 9·2 years) presenting with warts during this period were selected as controls.

Birth dates were grouped both by calender months and by astrological birth signs.

No significant overall difference was noted between the eczema and the control groups. However, for boys with eczema there was a significant preponderance of patients born from July to December versus those born from January to June (77/51; $P < 0{\cdot}05$). Similarly, more boys were born from Cancer to Sagittarius than in Capricorn to Gemini inclusive (80/48; $P < 0{\cdot}01$). No such association was seen in girls with eczema or in either sex with warts.

Thus, for boys, there appears to be an increased risk of atopic eczema in those born between the summer and winter solstices.

Clearly, further work is needed to relate parental birthsigns to our findings and to expand our study. Nevertheless, dermatologists may feel that there is sufficient evidence to advise parents with one atopic child to plan future conception so as to avoid these potentially hazardous celestial birth dates.

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## Marie-Unna syndrome

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Marie-Unna syndrome is a rare inherited disorder of hair growth. We have studied a North Derbyshire family in which the condition has affected over 68 members through seven generations. Affected individuals can be identified at birth by their coarse, fuzzy hair. Through childhood and adolescence there is progressive loss of hair from the crown of the scalp leaving a fringe around the margins. The final degree of hair loss varies considerably and may be total. The bald scalp has a scarred appearance and any surviving hairs are distorted and deeply pigmented. Body hair is sparse; the nails are normal.

Histological examination of the scalp using transverse sections revealed loss of normal hair follicles but with some persisting as small, often cystic, remnants. Scanning electron microscopy of hairs from four members of the family showed a number of abnormalities. These included longitudinal fluting, pili torti and a curious deformity resembling a knobbled tree branch.

This is the largest kindred of Marie-Unna syndrome described to date. The inheritance is by an autosomal dominant gene with a high degree of penetrance.

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## The cutaneous noduler chain: an unusual pattern of infection

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Discrete cutaneous nodules in an ascending chain along a limb are usually described with mention of sporotrichosis. Two patients with this sign presented to us within the space of one month; neither had sporotrichosis.

The first patient, a 38-year-old male hospital biochemist, and keeper of tropical fish, developed a chain of cutaneous nodules on the left hand, several weeks after a minor cut on the left ring finger. Biopsy of one of the nodules yielded *Mycobacterium marinum* on culture, and the lesion eventually resolved after prolonged treatment with oral Co-trimoxazole.

The other patient, a fit 68-year old gentleman, developed several tender inflammed cutaneous nodules in a linear fashion up the right hand and arm on return from a holiday in Florida. This was associated with pyrexia and suppuration, and was compatible clinically with staphylococcal lymphangitis, but failed to respond to appropriate parenteral antibiotics. Culture of fresh biopsy material subsequently yielded *Nocardia asteroides*, and he too was treated successfully with oral Co-trimoxazole.

Differential diagnosis of the 'Sporotrichoid' syndrome is discussed, and the term 'cutaneous nodular chain' suggested as more informative than 'sporotrichoid'.

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## Allergic contact dermatitis from an epoxy compound in a cutting oil

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Sensitization to additives in cutting oils is rarely reported. If positive patch tests to non-irritant dilutions of suspected cutting oils are obtained, further patch tests with the individual components of the oils are necessary to elucidate the exact allergen or allergens.

We have investigated five machine tool operators who have developed an occupational dermatitis while working with a particular cutting oil. Positive patch tests were first obtained to the oil at 50%, 25% and 10% in methyl ethyl ketone. Further patch tests with the various constituents of the formulation showed positive reactions to the chlorinated paraffin component. Subsequent tests with the constituents of this component showed the allergen in all five patients to be the glycidyl ester of hexahydrophthalic acid. This epoxy compound is used in the cutting oil to scavenge free chlorine ions and hence stabilize the oil. Only two out of five patients demonstrated cross-reactions with epoxy resin (1% petrolatum).

## Effects of topical and oral salbutamol therapy in atopic dermatitis

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The beta-2-adrenoceptor agonist, salbutamol, has been reported to have anti-inflammatory effects in the skin of both experimental animals (Seely & Glenn, 1978) and man (Archer *et al.*, 1984). The aim of this study was to assess the clinical effects of topical and oral salbutamol in atopic dermatitis and to discover whether salbutamol ointment therapy resulted in systemic absorption.

A randomized, double-blind, placebo-controlled study was performed, in which 21 patients with chronic atopic dermatitis were treated for 2 weeks with either (i) salbutamol (1% base) in white soft paraffin (WSP) b.d. plus placebo spandet b.d.; (ii) salbutamol slow-release spandet (8 mg) b.d. plus placebo ointment (WSP) b.d., or (iii) placebo spandet b.d. plus placebo ointment b.d. Clinical evaluations were made prior to the trial and on days 7 and 14 by giving a score of 0–3 (none, mild, moderate, severe) for redness, epidermal change, vesiculation and lichenification in each of 20 zones. Heart rate, blood pressure and airways function were measured at each visit and any side-effects noted. Plasma and 24 h urinary levels of salbutamol were estimated.

Salbutamol ointment therapy resulted in a reduction in the score for redness ($P < 0{\cdot}05$), but there was no marked clinical improvement in any of the treatment groups. Patients receiving salbutamol spandets experienced hand tremor which was related to plasma and urinary levels of salbutamol. Treatment with salbutamol ointment did not result in detectable plasma levels of salbutamol but there was evidence of systemic absorption in two patients, as demonstrated by significant urinary salbutamol levels. Clinical studies in atopic dermatitis using beta-2-adrenoceptor agonists with improved skin penetration would be of interest.

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## The use of itraconazole in chronic *Trichophyton rubrum* infections

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'Dry' type infections of the palms and soles, onychomycosis and certain forms of tinea corporis caused by *T. rubrum* are often refractory to treatment with griseofulvin or ketoconazole. Itraconazole, a new orally active triazole, shows good *in vitro* activity against *T. rubrum* (Espinel-Ingroff, Shadomy & Gebhart, 1984), and was used in this study to treat 22 patients with chronic dermatophytosis in an oral dose of 100–200 mg daily. The mean periods for achieving clinical and mycological remission for tinea corporis (10 patients) were 9·8 weeks and for infections of the palm (6 patients) or sole (6 patients), 7·8 and 12·6 weeks, respectively. Complete remission of onychomycosis of the finger nails was achieved in three patients (100%) and toe nails in two patients (50%). No clinical or biochemical abnormalities were detected during treatment, apart from mild abdominal discomfort in one patient. Within 10 weeks of stopping therapy, relapses have been confined to four patients in the tinea corporis group. All *T. rubrum* isolates tested ($n = 10$) were sensitive to a mean concentration of 0·6 μg/ml of drug (maximum 2·0).

Itraconazole appears to be an effective alternative treatment for recalcitrant *T. rubrum* infections.

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## Further clinical and investigative studies of chlorpromazine phototoxicity

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The precise wavelength dependency for chlorpromazine (CPZ) phototoxicity in humans ingesting the drug is not clearly established.

Seven subjects taking oral CPZ (dose range 100–500 mg per day, duration 2–48 weeks) were questioned, examined, and then phototested using solar simulator and irradiation monochromator sources. The clinical history of immediate, burning erythema (five subjects) induced by direct or window glass transmitted sunlight, and in some cases, a delayed erythema persisting for 24–48 h, established the reading times for the phototests.

Testing revealed that immediate erythema was the main abnormality, involving long wavelength ultraviolet (UV-A) and visible wavelengths (335–400 nm ± 30 nm; five subjects). Within the same waveband delayed erythema was less evident, affecting only two subjects. UV-B sensitivity was normal. Drug dose reduction with photoprotective clothing and zinc-oxide-containing cream reduced both severity and incidence of photosensitivity.

In laboratory studies, CPZ-photosensitized damage to lymphocytes was obtained with drug concentrations ($>5\ \mu g\%$), UV-A exposures (3 $J/cm^2$) and conditions consistent with genuine photosensitization processes. This model may be more useful for elucidating the mechanisms of cutaneous phototoxicity due to CPZ than photohaemolysis or the photosensitized killing of macrophages, for which a toxic photoproduct is responsible, and the most active wavelengths are in the UV-B range.

## Phototoxic plants in Britain

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In the U.K., four families of plants are known to induce phytophotodermatitis in man. These families are the Apiaceae (Umbelliferae), including the giant Russian hogweed, the Rutaceae, including rue, the Moraceae (figs), and the Papilionaceae, or pea family. The important botanical features of these families are illustrated.

In general, the phototoxic furocoumarins are found in the healthy plant. More rarely, they may be synthesized by the plant in response to fungal invasion (parasito-phytophotodermatitis), or even produced by the parasitic fungi themselves. These substances may develop after prolonged storage or inappropriate handling.

Recognition of the major plant families is important, and not difficult.

## Micro-computer-based system for contact dermatitis

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*Department of Dermatology, Wycombe General Hospital, *The Skin Hospital, Manchester and †Barclay Microprocessor Group, University of Manchester*

Patch testing generates two different types of data. These are: patient related—information which is of direct help in managing an individual case—and comparative patch test data in respect of the incidence of allergy to particular allergens in the environment and their relationship to occupation, primary site of dermatitis, age, sex, race, and other factors.

This latter type of information is very difficult and time-consuming to extract by hand, and is ideally suited for analysis by a microprocessor.

We discuss the factors that influence choices of hardware, software and the programming necessary to achieve a comprehensive data base. A standardized list of occupations, hobby groups and allergen groups have been developed, as well as a disease classification of the various eczema and non-eczema groups normally submitted for patch testing.

Examples of how computer analysis of patch test results can be used are given.

## Attitudes to additives—a preliminary report

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As part of a 3-year survey commissioned by the Ministry of Agriculture, Fisheries and Food, our department has investigated food additive intolerance by means of a questionnaire sent to 10% of the Wycombe Health Authority population, i.e. 30 000 people in 11 000 households in our area.

The questionnaire was designed in collaboration with the British Market Research Bureau to identify those in the population who thought they had food additive intolerance. We wished to identify the association of 'food additive intolerance' with food intolerance, salicylates/NSAI intolerance, and atopic disease.

Our main objective is to identify those who react to food additives with urticaria/oedema. These patients will form part of a major study with the Department of Medicine, Guy's Hospital/British Industrial Biological Research Association to elucidate the mechanisms and pharmacology of the condition. The incidence of skin disease has been specifically enquired for, as have all other symptoms reported in the literature as being possibly related to food additives.

The prevalence of food additive intolerance is stated to be 0·03–0·15% of the population (M.A.F.F. figures). Our questionnaire achieved a 62% response, and 7·2% of the respondent population stated that they had a problem with food additives.

We present our preliminary findings with particular regard to perceived dermatological symptoms related to food additives, the role of food additives in hyperactivity, and the association with atopic disease.

## Solitary morphoea profunda

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This study describes a series of patients with a hitherto unrecognized form of morphoea. All four patients presented with large, solitary, indurated plaques on the upper back. Atrophy was not a feature clinically and two cases showed a peau d'orange effect. Histology revealed extensive sclerosis of the reticular dermis and subcutaneous tissue, and an inflammatory cell infiltrate which was located predominantly at the level of the subcutis. Numerous plasma cells were present in all cases and in three cases formed the predominant cell type. Eosinophils were present in one case only. Lymphoid aggregates were prominent and caused some diagnostic confusion in one case, which was initially reported as a lymphoma. Follow-up for periods up to 12 years has revealed no evidence of systemic involvement. Only one patient has developed further lesions.

Involvement of the subcutaneous tissue is well described in cases of generalized morphoea (Fleischmayer, 1972) but the series of 23 patients reported by Person and Su (1981) under the title of morphoea profunda included only one case of solitary morphoea. The clinical and pathological features of our cases seem sufficiently distinct to merit description under the title Solitary Morphoea Profunda.

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## Oral nifedipine treatment for Raynaud's phenomenon secondary to systemic sclerosis—a double-blind, placebo-controlled study

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Oral nifedipine has been advocated as treatment for Raynaud's phenomenon (Smith & McKendry, 1982); however, previous studies have been of only 2–3 weeks duration and have included patients with Raynaud's phenomenon of varied aetiology. Furthermore, it has been suggested that the Raynaud's phenomenon experienced by systemic sclerosis (SS) patients responds less favourably to nifedipine than does idiopathic Raynaud's disease.

To assess the efficacy of nifedipine in Raynaud's phenomenon secondary to SS, we have performed a double-blind, placebo-controlled, crossover, winter trial of nifedipine 10 mg thrice daily for 6 weeks in 10 patients with SS. Nine patients completed the study.

Patients experienced a significant reduction in duration of Raynaud's attacks following nifedipine treatment compared with placebo ($P < 0{\cdot}01$; Wilcoxon signed ranks test). A small decrease in the number and severity of Raynaud's attacks and the number of new digital ulcers was also observed, and a slight increase in digital blood flow was measured (strain-gauge and photo-plethysmography), although not achieving statistical significance.

Neither red blood cell deformability, nor whole blood or isolated polymorph chemiluminescence was modified by nifedipine treatment.

This study has demonstrated that nifedipine evokes a small degree of improvement in the Raynaud's phenomenon experienced by patients with systemic sclerosis.

### REFERENCE

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## Cutaneous endometriosis: a histopathological study

M.J.TIDMAN AND D.M.MACDONALD

*Department of Dermatology, Guy's Hospital, London*

Ectopic endometrial tissue within the skin is an unusual occurrence and may cause diagnostic difficulty. The detailed histopathological characteristics of cutaneous endometriosis were studied in material from 10 cases, presenting with dermal or subcutaneous nodules, arising either spontaneously within the umbilicus or associated with abdominal scars related to uterine surgery.

The endometriomas consisted of glandular elements embedded in a vascular and cellular stroma containing foci of intense erythrocyte deposition. Features reminiscent of the uterine endometrium at each of the main phases of the menstrual cycle were observed, although in those 40% of cases for whom precise menstrual details were available, there existed a poor correlation between histological appearance and menstrual stage. The proliferative phase was suggested by a uniform stromal cell population and pronounced epithelial mitotic activity, and the secretory phase by evidence of decapitation secretion within the glandular cells and the presence of two stromal cell types, morphologically analogous to the 'predecidual cell' and 'endometrial granulocyte' of the uterus. Disintegration of the epithelium and dissociation from the stroma resembled menstruation.

The 'endometrial granulocyte' and clear cells within the columnar lining of the glandular lobules both possessed small hyperchromatic nuclei and abundant cytoplasm containing refringent phloxinophilic granular material, suggesting that these two cell populations are identical.

## Epidermal Class II HLA expression in atopic dermatitis

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To test the hypothesis that epicutaneous antigen may play a role in atopic dermatitis (AD), we have studied epidermal Class II HLA expression in lesional atopic skin. Class II antigens, the

HLA-D series, are expressed only on certain cells, and their expression is thought to be a prerequisite for any cell to act as an antigen presenting cell. In allergic contact dermatitis (CD), as well as in other dermatoses where allergen contact is thought to play a role, keratinocytes have been found to express HLA-D, suggesting that they may act as antigen presenting cells. If similar mechanisms occur in AD, keratinocyte HLA-D expression might be expected.

Using an indirect immunoperoxidase technique, we have studied 20 AD patients with a panel of monoclonal antibodies recognizing each separate Class II sub-region -DR, -DQ and -DP. Positive staining was noted in the dermal infiltrate and on epidermal dendritic cells. Keratinocytes were uniformly negative with each antibody used. Positive staining in CD was confirmed.

Our results tend to suggest that the immune response to epicutaneous antigen may not be responsible for AD lesions. However, it is possible that early in the pathogenesis of the lesions, keratinocytes may express Class II antigens, but that these are lost with time.

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## The detection of extracellular antigens in formaldehyde-fixed paraffin-embedded cutaneous tissue

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In the investigation of patients with suspected bullous disease, lupus erythematosus and vasculitis, conventional processing of skin biopsies prevents the detection of many extracellular antigens, particularly immunoglobulins. Consequently, fresh frozen specimens are required for routine immunofluorescence (IF). Disadvantages of this method include poor preservation of morphological detail, the necessity to anticipate freezing the tissue at the time of biopsy, storage problems and lack of permanent preparations.

We have developed a modified formaldehyde-fixed paraffin embedding technique which allows the detection of immunoglobulins and complement in the above mentioned disorders. Employing both direct and indirect IF and immuno-enzymatic (peroxidase-antiperoxidase) methods, the immunoreactivity achieved on processed tissue was comparable with results obtained on fresh frozen cryostat controls.

The method involves limited fixation in formal saline for 4 h, limited dehydration and clearing at 4°C and wax embedding at 58°C for 1 h. Antigens are 'unmasked' by pre-treating the dewaxed sections with pronase. This processing procedure uses routine materials and, additionally, produces sections which can be stained conventionally with haematoxylin and eosin. The technique provides superior morphological preservation over frozen sections and permanent sections suitable for indefinite storage and restrospective studies.

## Treatment of common and plantar viral warts with human lymphoblastoid interferon-α

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Interferons possess antiviral, antiproliferative and immunomodulatory properties and thus appear to be a rational choice as a therapy for severe, persistent warts which prove resistant to conventional therapy. The aims of these open studies were to evaluate the relative merits of the intralesional, intramuscular and dermojet routes of administration, and to establish a safe, effective and patient-acceptable treatment regime which could subsequently be used in fully controlled studies.

Human lymphoblastoid interferon-α (Wellferon, Wellcome Foundation Ltd) has been used in all the studies. The minimum criteria for entry into the studies were the presence of common and/or plantar viral warts for at least one year which were resistant to conventional therapies.

Intramuscular therapy given to 17 patients in divided doses totalling 5·6–14 mega units per week for periods of up to 19 weeks have yielded modest results, with approximately 30% of patients showing complete or significant clearance of their warts. However, intralesional injections given once weekly to 16 patients in doses ranging between 1·1 and 5·8 mega units for periods usually not exceeding, and sometimes less than, 12 weeks have been remarkably successful, with more than 90% of patients who completed their treatment course showing full clearance of the injected warts. The dermojet data are preliminary but moderately encouraging.

The intramuscular treatment regime was associated with influenza-like symptoms in virtually all patients and a significant drop in the white cell count in approximately one third of patients. Intralesional injections are momentarily painful and are associated with influenza-like symptoms, but are remarkably free from clinically apparent local tissue reactions and do not appear to have effects on haematological or biochemical parameters measured.

It would appear that the intralesional route is the most practical one as it combines a reasonable therapeutic ratio with a procedure that may be performed quickly and with no prior preparation of the lesion.

## Clinical and laboratory manifestations of cutaneous virilism

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The concept of primary cutaneous virilism has been used to explain the integumentary signs seen in many women who are otherwise healthy (Shuster, 1972); its basis lies in the finding of increased skin metabolism of androgens in hirsutism and acne. Many other cutaneous signs, which may occur in isolation or variously grouped, have been considered as androgen dependent.

We have therefore studied 38 hirsute women aged 16–39 years (median 27 years) by clinical and laboratory assessment with regard to cutaneous and systemic hyperandrogenism. Hirsutism was graded objectively, using the Ferriman and Gallwey (1961) scale, at 17–40 (median 27).

Significant associations found in the subjects included acne vulgaris (60%) average grade 2 (1–5), keratosis pilaris in 16 (42%), acanthosis nigricans in 10 (26%) and evidence of hydradenitis suppurativa (comedones, papules or cysts in apocrine sites) in 11 (29%). Scalp hair pattern revealed Ludwig (1977) type 2 diffuse alopecia in only two subjects. Body morphology using the standard waist-hip ratio demonstrated a tendency to a male pattern of fat distribution (normal women 0·73, hirsute women 0·80, normal men 0·88). Sebum excretion rate (Strauss & Pochi, 1961), maximum sweat rate (pilocarpine iontophoresis) and testosterone/sex hormone binding globulin ratio were all raised.

The presence of all these changes in hirsutism broadens the concept of androgen-mediated skin signs which may have therapeutic implications with the advent of new and potentially less toxic anti-androgen drugs.

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## Contact sensitivity in community-based leg ulcer patients

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Leg ulcers are a chronic and disabling disease affecting approximately 0·1% of the population. The majority of leg ulcer patients in the U.K. are cared for by community nurses. Complications such as failure to heal, infection and acute contact dermatitis bring them to the attention of a dermatologist.

Patients attending leg ulcer clinics in Oxford are routinely patch tested to exclude medicament allergy. The incidence of contact sensitivities is 81%, the commonest allergens being neomycin and framycetin, wood tars, wool alcohols, coal tar and fragrance mix. For the majority of leg ulcer patients based in the community, this service is not available and therefore the incidence of medicament allergies and the morbidity they cause, is not known.

The object of this study was to determine the incidence of contact sensitivity in community leg ulcer patients and to see if we could identify any repetitive pattern or group of patients who became sensitized. Thirty-six patients (27 women and 9 men) from seven different practices in the Oxford region were patch tested with 28 different substances. 19 patients (53%) showed one or more positive reactions; 17 (47%) patch tests were negative. The commonest allergens, similar to hospital experience, were wool alcohols, parabens neomycin and framycetin, fragrance mix and ester gum resin.

Both patients who had gravitational eczema and leg ulcers, and those who were eczema-free, had positive patch tests, but the incidence was higher in those with eczema. The length of the clinical history did not correlate with increased incidence of allergies, nor did the presence of more than one ulcer, or ulcers on both legs.

In 15 of 36 patients with positive reactions, these sensitivities were of present relevance. In the remainder, four were of past relevance. This study demonstrates an incidence of contact sensitivity in community patients of 50%. Ideally, all leg ulcer patients should be patch tested. Our experience indicates that, if facilities are limited, patch testing should be offered to those patients who have gravitational eczema as well as their leg ulcers.

### Ehlers-Danlos syndrome type VIII

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The Ehlers-Danlos syndrome is a group of inherited disorders of connective tissue; several distinct sub-groups have been described, each with characteristic clinical features of a specific biochemical defect. Three cases are described with the diagnostic clinical manifestations of Ehlers-Danlos type VIII syndrome. A unique biochemical defect of collagen metabolism was documented in this sub-group.

Each family demonstrated an autosomal dominant pattern of inheritance. Early onset of periodontal disease with loss of permanent teeth, easy bruising and typical hyperpigmented, atrophic pretibial scars following minor trauma in childhood were characteristic. Cutaneous hyperextensibility and joint hypermobility were either minimal or absent; there was no evidence of visceral involvement.

Histopathology of the skin lesions was also typical showing dermal atrophy with scarring and focal fragmentation, and clumping of elastic fibres in the reticular dermis.

Skin fibroblasts from normal subjects and patients with Ehlers-Danlos type VIII were treated with supernatants from monocyte cultures to determine whether secretion of collagenase could be stimulated. Monokine stimulated skin fibroblasts from the Ehlers-Danlos type VIII patients produced elevated levels of collagenase activity, compared to controls, whilst there was no detectable collagenase in unstimulated cultures.

*British Journal of Dermatology* (1986) **115,** Supplement **30,** 43–104.

# CASE REPORTS

Unless otherwise stated, cases were seen at The Royal Hallamshire Hospital, Sheffield

## (1) Focal facial dermal dysplasia (Brauer's syndrome)

DR C.I.HARRINGTON

K.B., male, aged 1 year.

*History*. This baby was born by normal delivery at 38 weeks gestation weighing 5lb 14oz. Purple scars were noted on his scalp and forehead at birth. His mother's pregnancy had been uneventuful with no drug ingestion or exposure to infection.

*Family history*. His mother is unmarried but has suggested that the paternal greatgrandfather may have been similary affected. Offers of genetic counselling have been declined.

*Examination*. When he was first seen at the age of 3 weeks, purple, scar-like atrophic lesions were present on his forehead (Fig. 1), temples, nose and cheeks. A few lesions were present on the abdomen. The atrophic areas are now fading and becoming less noticeable. His scalp hair and eyebrows are sparse but he is otherwise completely normal.

*Comment*. This case fits the description by Brauer (1929) of naevus aplasticus, but is more suitably designated focal facial dermal dysplasia (McGeogh & Reed, 1971).

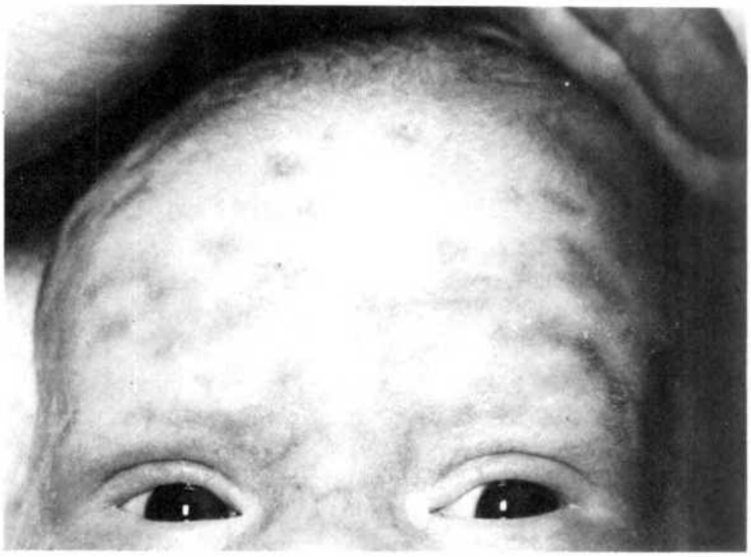

FIGURE 1. Atrophic 'scar-like' lesions on the forehead as seen at the age of 3 weeks.

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## (2) Focal dermal hypoplasia (Goltz's syndrome)

DRS S.B.BITTINER AND R.E.CHURCH

M.A., female, aged 20 years.

*History.* A bullous eruption was present at birth periorally, on the left side and on the left thigh. No drugs had been administered during the pregnancy. The birth was a normal delivery at 39 weeks, birth weight 5lb 7½ oz. Five days after birth there was a well-defined denuded area of skin on the left side, extending from the costal margin to the iliac crest. There were similar lesions around the mouth and on the anterior aspect of the left thigh. There were two small herniae of the umbilicus, fusion of the right 3rd and 4th fingers, with absence of the right middle finger nail, and partial fusion of the 3rd and 4th toes of the right foot. In 2 weeks the lesions were covered by a layer of epidermis, but remained depressed below the level of the surrounding skin. At the age of one year the skin lesions broke down and the epidermis was shed. From the bed of each lesion arose small granulation-like papules. These became covered with epidermis on the leg, but subsided spontaneously on the lesion of the trunk. Since then the epidermis has remained intact apart from an area on the left flank.

*Examination.* The patient is thin and of small stature. The facies have a pinched appearance. There is telangiectasia of the face and areas of atrophy. On the left thigh there is an area of multiple fat herniations. The finger nails are small and ridged.

*Investigations.* In 1970, the growth hormone level was elevated.

*Histology.* There is absence of dermal collagen, with subdermal fat reaching through to the epidermis.

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GOLTZ, R.W., HENDERSON, R.R., HITCH, J.M. & OTT, J.E. (1970) Focal dermal hypoplasia syndrome. *Archives of Dermatology*, **101**, 1.

## (3) Focal dermal hypoplasia (Goltz's syndrome) with extensive capillary haemangioma

DR S.S.BLEEHEN

V.P., female, aged 11 years.

*History.* Extensive capillary angioma was noted at birth. In 1980, the patient was referred for a further opinion. The clinical features and the multiple developmental defects were those of Goltz's syndrome.

*Family history.* An elder sibling is normal, but the mother has an abnormal dentition.

*Examination.* There are many foci of dermal hypoplasia with several soft nodules on the limbs, with herniation of the fat. An extensive capillary haemangioma affects the right arm and upper chest (Fig. 1). In addition, there are linear streaks of telangiectasia. There is syndactyly of the toes and brittle thin nails. The teeth are poorly developed and there is a patchy alopecia.

*Skin biopsy* (14.1.81). The dermis is extremely thin and the subcutaneous fat is close to the epidermis.

*Comment.* This case is typical of the syndrome as described by Goltz *et al.* (1962), although it is unusual in that there is an extensive haemangioma in addition to the linear streaks of telangiectasia.

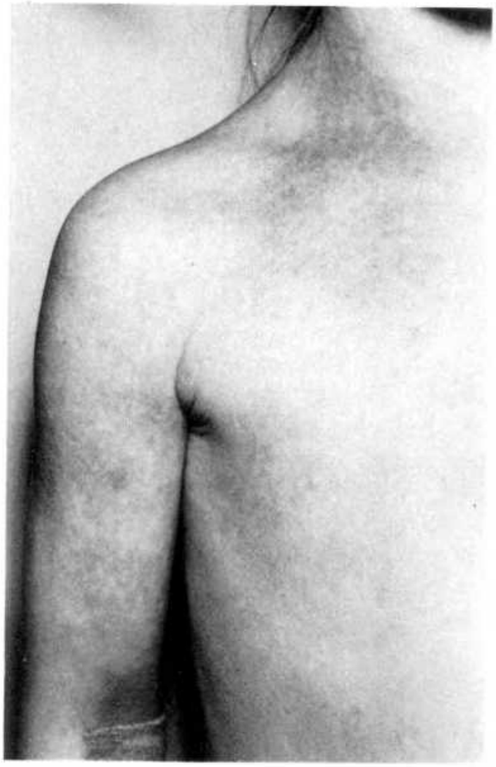

FIGURE 1. Extensive capillary haemangioma with areas of dermal hypoplasia on the right side of the chest and arm.

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GOLTZ, R.W., PETERSON, W.C., GORLIN, R.J. & RAVITS, H.G. (1962) Focal dermal hypoplasia. *Archives of Dermatology*, **86**, 708.

## (4) Tricho-rhino-phalangeal syndrome

DRS S.E.THOMAS, J.MAHOOD AND R.E.CHURCH

S.B., male, aged 24 years.

*History*. All his life, the patient had noticed that his scalp hair grew rather slowly, requiring haircuts, on average, once every 4 months. He started to 'go bald' at 14 years of age, beginning on the crown. For several years he had noticed a progressive painless deformity of several finger joints. As a child he received dental treatment for 'extra front teeth'. There was no relevant family history.

*Examination*. Most of his scalp hair had been replaced by vellous-like hair and he had a rather prominent nose with a long philtrum (Fig. 1). Slight notching of the 2nd upper incisors was

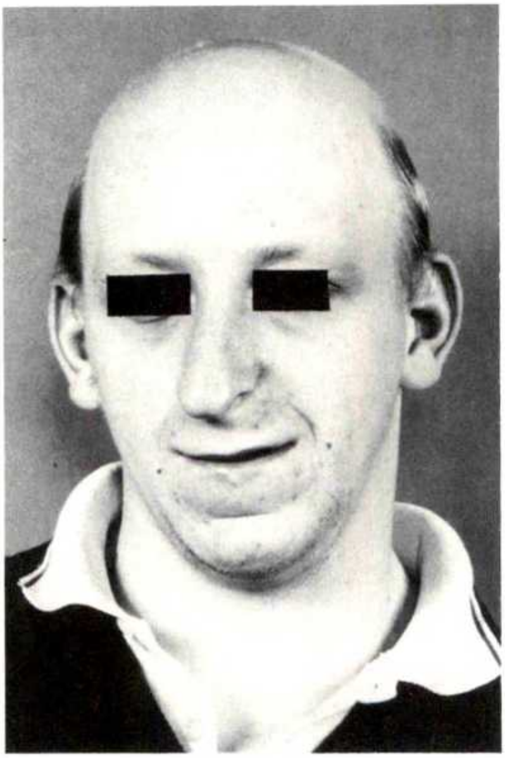

FIGURE 1. Facial features showing loss of scalp hair and a prominent nose.

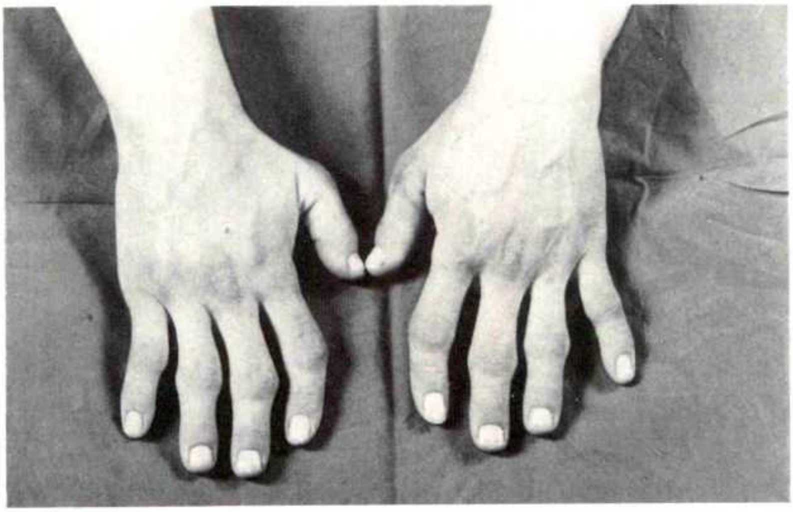

FIGURE 2. Ulnar deviation of the proximal inter-phalangeal joints.

present. There was ulnar deviation of the proximal inter-phalangeal joints, and most fingers had some joint swelling present (Fig. 2). The nails showed marked leukonychia.

Clinical examination was otherwise normal.

*Investigations.* The chest X-ray and skull X-rays were normal. X-rays of the hands and feet revealed shortening of peripheral phalanges, 1st metatarsals and metacarpals. There are cone-shaped phalangeal epiphyses. The appearances are those of a peripheral dysostosis and are consistent with the tricho-rhino-phalangeal syndrome.

*Comment.* This patient shows all the clinical and radiological features described previously in the syndrome. A similar case was presented at the Summer B.A.D. Meeting in 1979. The inheritance has yet to be established, and no apparent biochemical defect has been discovered.

### REFERENCE

GREDON, A. (1967) Cone-shaped epiphyses. The tricho-rhino-phalangeal syndrome. *Annales de Radiologie*, **10**, 322.

## (5) Incontinentia pigmenti. Bloch-Sulzberger syndrome

DRS S.S.BLEEHEN AND M.L.WOOD

*Childrens Hospital, Sheffield*

F.F., female, aged 11 years.

*History.* Within a few hours of being born, the patient developed a widespread erythematous vesicular rash on the trunk and limbs. Later, streaks of hypermelanosis were noted. At that stage, there was a marked eosinophilia in the peripheral blood. At the age of 2 months, there were lichenoid and warty lesions present, particularly on the legs. Over the years, the areas of hypermelanosis have become less apparent, but still persist.

*Family history.* One brother is normal.

*Examination.* Several slatey-brown hypermelanotic streaks are present on the lower legs and abdomen. Some loss of hair is apparent on the scalp. The right eye is smaller than the left and is sightless. There are several large, white patches present in the retina representing retinal dysplasia. There is a marked defect of dentition with absent teeth and several that are peg-like.

*Skin biopsy.* A skin biopsy was performed at the age of 4 days. This showed intra-epidermal vesicles with eosinophilic spongiosis and several dyskeratotic cells. Increased numbers of mast cells were present in the upper dermis.

*Comment.* This child has the typical cutaneous lesions of incontinentia pigmenti of the Bloch-Sulzberger type. She presented with widespread erythema with vesiculation and later developed warty lichenoid lesions. She still has a number of slatey-brown splashes of hyperpigmentation due to pigmentary incontinence. Ocular defects as seen in this child are not uncommon and are found in about 30% of cases (Carney & Carney, 1970). In addition, she has several dental abnormalities.

### REFERENCE

CARNEY, R.G. & CARNEY, R.G. JR. (1970) Incontinentia pigmenti. *Archives of Dermatology*, **102**, 157.

## (6) Incontinentia pigmenti achromians (hypomelanosis of Ito)

DRS S.S.BLEEHEN, S.B.BITTINER AND B.L.PRIESTLEY

*Children's Hospital, Sheffield*

R.A., male, aged 10 years.

*History*. The patient was referred to hospital at the age of 9 months because of substantial delay in his motor development. It was noted that he was 'floppy', with marked hypotonia. He was later slow to speak and walk. At the age of 4 years pigmentary changes were noted.

*Family history*. His elder brother is normal. There is no significant family history.

*Examination*. There are hypomelanotic streaks and whorls on the trunk and limbs (Fig. 1). He shows mild facial dysmorphism and has bilateral transverse palmar creases. There is a supernumerary accessory digit on the left palm. The head circumferance is on the 98th centile. There is persisting, marked hypotonia, poor co-ordination and clumsiness. He has a severe degree of hearing loss, worse since an attack of chicken pox.

*Investigations*. Creatine phosphokinase, urinary chromatogram for amino acids and chromosome analysis were all normal.

*Skin biopsy* (18.3.81). There is a patchy loss of pigment in the basal layer of the epidermis. Melanocytes are present and their morphology on electron microscopy appears normal.

*Comment*. This boy shows the typical streaks and whorls of hypomelanosis seen in incontinentia pigmenti achromians of Ito. There is a high incidence of neurological abnormalities in this disorder (Schwartz *et al*., 1977; Takematsu *et al*., 1983), as observed in this case.

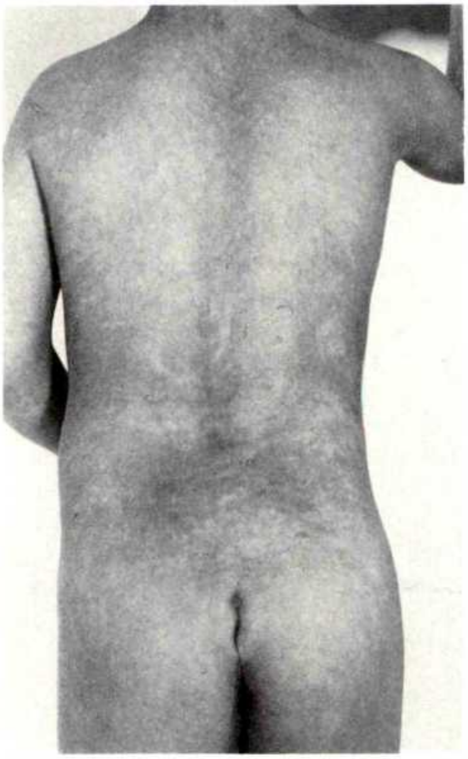

FIGURE 1. Streaks and whorls of hypomelanosis on trunk.

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TAKEMATSU, H., SATO, S., IGARASHI, M. & SEIJI, M. (1983) Incontinentia pigmenti achromians (Ito). *Archives of Dermatology*, **119**, 391.

## (7) Klippel-Trenaunay-Parkes-Weber syndrome

DRS W.F.G.TUCKER AND C.I.HARRINGTON

N.M., male, aged 8 years.

*History*. At birth, this boy was noted to have an extensive vascular naevus affecting the right side of his face and body. As he has grown, his right arm and right leg have become noticeably larger than their counterparts.

*Examination*. Examination reveals an extensive capillary naevus affecting the right side of the face and neck, the right arm, much of the right side of the trunk and large areas of the right leg. There is obvious enlargement of his right hand and arm and the right leg. (Fig. 1).

*Investigations*. Chest and skull X-rays were normal. An EEG showed no evidence of paroxysmal activity.

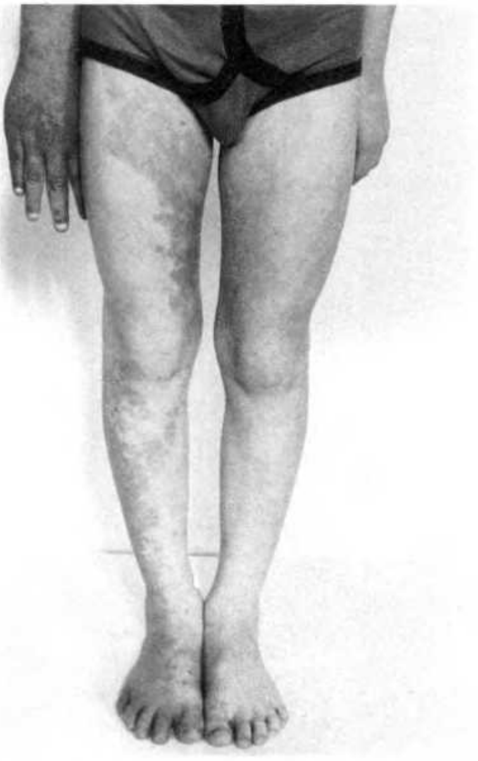

FIGURE 1. Extensive capillary naevus and prominent limb enlargement.

*Treatment*. No orthopaedic intervention is planned in the immediate future. Dr Cotterill has kindly agreed to assess this young man with regard to future laser treatment. Covermark has recently been tried and pronounced a success by the patient.

*Comment*. No satisfactory treatment has yet been reported for the underlying deep vascular abnormalities, but we are hopeful that the laser treatment to his face and hand will improve his appearance.

## (8) Cutis marmorata telangiectatica congenita

DRS W.F.G.TUCKER AND S.S.BLEEHEN

*Children's Hospital, Sheffield*

G.R., female, aged 2 years.

*History*. This infant was noted at birth to have unusually prominent vascular markings of the right arm and leg. When first seen in the skin clinic one month later, the appearances of cutis marmorata were noted to affect the right forearm, right side of the back and the flexor surface of the right leg. Atrophy of the subcutaneous tissues of the right forearm and the dorsum of the right hand was noted by the age of 5 months. There has been no change in the extent of the lesions.

*Examination*. Prominent reticulate vascular patterning is present over the dorsum of the right hand and forearm (Fig. 1), the right side of the upper back and the flexor aspect of the right calf

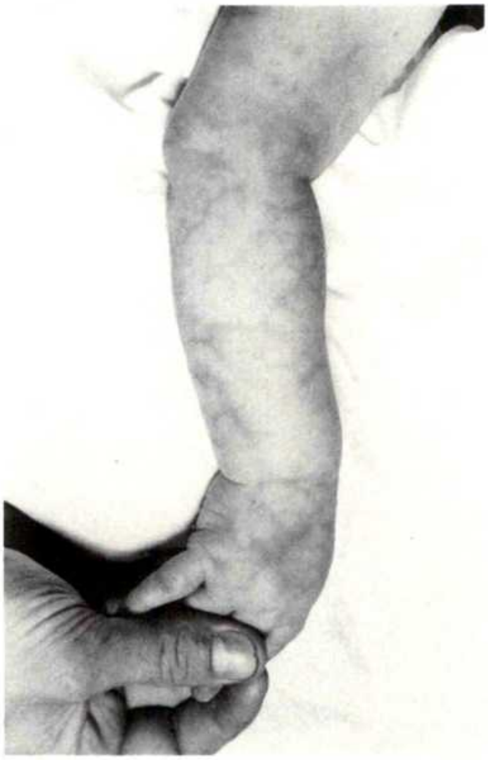

FIGURE 1. Reticulate vascular patterning on right arm when aged 6 months.

and thigh. There is an apparent partial absence of the subcutaneous fat of the right forearm and, particularly, the dorsum of the right hand. There is no asymmetry in the length of the arms or legs and no underlying vascular anomalies have been detected. The cardiovascular and central nervous systems appear normal.

*Progress.* Although the reticulate vascular patterning is obvious in cold weather, the lesions appear to be gradually fading and there has been a slight improvement of the subcutaneous atrophy over the past year. The child has no problems in using the right arm and is developing normally.

*Comment.* Cutis marmorata telangiectatica congenita, as the name implies, is characterized by an exaggeration of the 'normal' transient cutis marmorata often seen in young infants, with generalized or localized reticulate mottling of the skin. Associated anomalies include venous varicosities and angiokeratomas, and occasional superficial ulceration may occur. Subcutaneous hypoplasia and hypertrophy may occur, together with hypertrophy of the underlying limb bone (Way *et al.*, 1974). Most children are otherwise normal, although some reported cases have had cardiac or neurological problems (Petrozzi *et al.*, 1970). The condition is more common in females, and both an autosomal dominant and a multifactorial inheritance have been proposed (Andreev & Pramatarov, 1979).

### REFERENCES

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## (9) Cutis marmorata telangiectatica congenita

DRS S.B.BITTINER AND R.E.CHURCH

M.A., female, aged 17 years.

*History.* Congenital atrophy of the skin was noted from birth. There was a marked improvement during the first year of life. The patient presented to the Royal Hallamshire Hospital in 1982 for cosmetic advice. Her general health is good. There is no relevant family history.

*Examination.* There is extensive reticulate livedo involving the trunk and limbs. The face is spared. There is prominent distension of the superficial veins of the legs, forming some venous lakes. The general physical examination is normal.

*Investigations.*The chest X-ray is normal.

*Treatment.* The patient is using make-up as camouflage.

*Comment.* We would welcome any suggestions regarding the management of this girl's extensive cutaneous abnormality.

### REFERENCE

WAY, B.H., HERMANN, J., GILBERT, E.F., JOHNSON, S.A. & OPITZ, J.M. (1974) Cutis marmorata telangiectatica congenita. *Journal of Cutaneous Pathology*, **1**, 10.

## (10) Alagille's syndrome (arteriohepatic dysplasia)

DRS D.M.LISTER, D.SHUTTLEWORTH, R.A.C.GRAHAM-BROWN AND S.TANNER

*Leicester Royal Infirmary*

S.S. and D.S., female, aged 7 and 8 years.

*History.* Both sisters suffered protracted neonatal jaundice and since then have had severe, persistent pruritus.

*Examination.* The girls show a characteristic facial appearance. They and their mother have retinal pigmentation (Fig. 1).

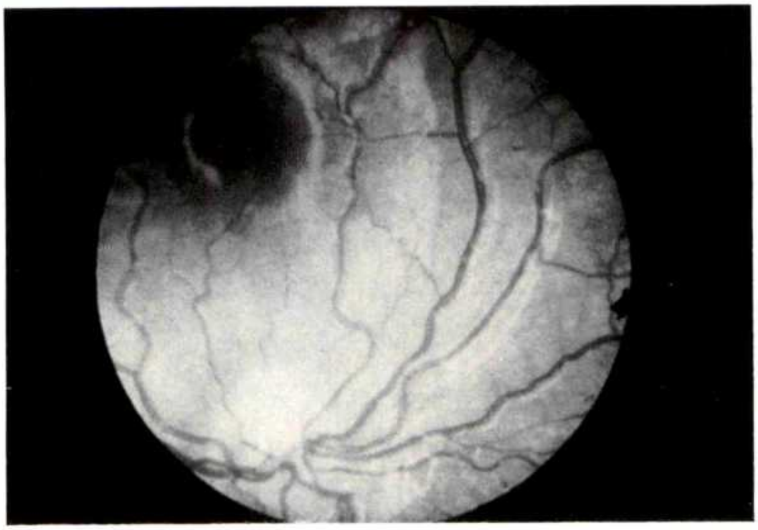

FIGURE 1. Marked retinal pigmentation, typical of Alagille's syndrome, is present.

*Investigations.* The sisters have elevated serum lipids. The bilirubin, originally elevated, is now normal in both girls. S.S. (the elder sister) has had a liver biopsy which showed the presence of intrahepatic biliary atresia. She has also been found to have a large atrial septal defect which has been closed successfully. Further investigations of their mother have been normal.

*Treatment.* Control of their pruritus has proved difficult. Both children have noticed some improvement with oral cholestyramine. Simple emollients have also been helpful but a course of ultra violet light failed to produce any improvement.

*Comment.* Alagille's syndrome is a rare, genetically determined condition inherited as an autosomal dominant trait with incomplete penetrance. Associated abnormalities may be found in the eyes, bones and cardiovascular system (Alagille *et al.*, 1975). Pruritus is a major symptom and may be the presenting feature of this unusual condition (Ryatt *et al.*, 1983). It is therefore important that dermatologists should be aware of its existence.

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RYATT, K.S., COTTERILL, J.A. & LITTLEWOOD, J.M. (1983) Generalized pruritus in a baby as a presenting feature of the arteriohepatic dysplasia syndrome. *Clinical and Experimental Dermatology*, **8**, 657.

## (11) Livedo reticularis and cerebrovascular lesions

DRS S.E.THOMAS, R.E.CHURCH AND G.A.B.DAVIES-JONES

F.W., female, aged 31 years.

*History*. In August 1981, the patient developed transient functional loss affecting the right arm. One month later, she suddenly developed a right hemiparesis and dysphasia. She was fully investigated in the department of neurology, and cerebral angiography revealed thrombosis of the left middle cerebral artery. In December 1984, she was re-admitted for re-assessment. She had been suffering transient loss of function of the left arm and the left side of her face during the previous 12 months.

In the past she had had three pregnancies; two stillbirths at 27 weeks gestation, and one live birth at 32 weeks. Her only child, now aged 4 years, has a congenital right hemiparesis.

*Examination*. Widespread broken livedo reticularis was apparent over the trunk and limbs (Fig. 1). The patient had a right hemiparesis and dysphasia. The blood pressure was 120/70 and clinical examination was otherwise normal.

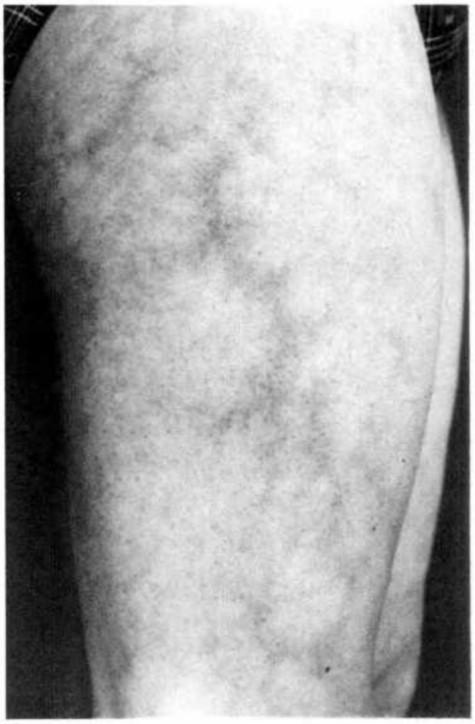

FIGURE 1. Prominent livedo reticularis on the leg.

*Investigations*. Haemoglobin, full blood count, ESR, ANF, anti-DNA antibody, immunoglobulins, complement, cardiolipin antibodies, VDRL and cryoglobulins were all normal. Lupus anticoagulant result is still awaited.

*Histology*. The skin biopsy is consistent with livedo reticularis. Direct immunofluorescence is negative.

*Treatment*. The current treatment is Aspirin® 75 mg daily.

*Comment*. Several reports have linked livedo reticularis with cerebral thrombosis. (Church, 1962; Sneddon, 1965). Cardiolipin antibodies and lupus anticoagulant have been described in association with cerebral thrombosis in systemic lupus erythematosus (Boey *et al*., 1983). Systemic lupus erythematosus remains a possibility in this case.

### REFERENCES

BOEY, M.L., COLACO, C.B., GHARAVI, A.E., ELKON, K.B., LOIZOU, S. & HUGHES, G.R.V. (1983) Thrombosis in systemic lupus erythematosus: Striking association with the presence of circulating lupus anticoagulant. *British Medical Journal*, **287**, 1021.

CHURCH, R.E. (1962) Reticular livedo with cerebrovascular lesions. *British Journal of Dermatology*, **74**, 156.

SNEDDON, I.B. (1965) Cerebrovascular lesions with livedo reticularis. *British Journal of Dermatology*, **77**, 180.

## (12) Discoid lupus erythematosus

DRS S.E.THOMAS AND R.E.CHURCH

J.C., male, aged 58 years.

*History*. For 30 years, this patient has been attending the dermatology department with

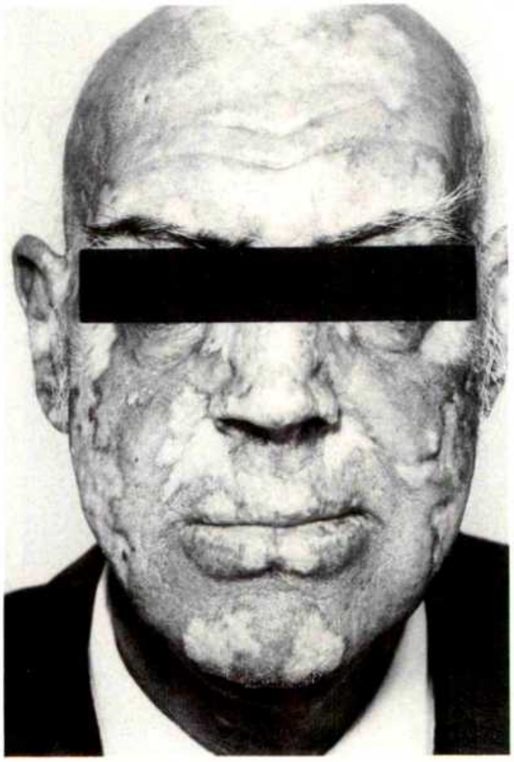

FIGURE 1. Extensive scarring and depigmenting lesions of discoid lupus erythematosus on the face.

severe chronic discoid lupus erythematosus. He had a right-sided mastoidectomy performed 40 years ago, and has suffered from pneumonoconiosis for 22 years.

*Examination.* Severe lesions of discoid lupus erythematosus affecting the light exposed areas on the face (Fig. 1), the 'V' of the neck, and the backs of the hands are present. Gross scarring and depigmentation has occurred as a result of these lesions. On examination of the chest there is a moderate degree of emphysema. Clinical examination is otherwise normal.

*Investigations.* Haemoglobin, full blood count, ESR, anti-doublestranded DNA antibody, rheumatoid factor, renal and liver function tests, and lipids are all normal. The chest X-ray showed a reticulo-nodular pattern throughout both lung fields consistent with pneumoconiosis.

*Histology.* The biopsy showed hyperkeratosis with keratin plugs in the follicles around which is an intense chronic inflammatory infiltrate, mainly lymphocytes. The basal cells show marked hydropic degeneration.

*Treatment.* For over 30 years, this patient has been treated with topical and systemic steroids, chloroquine, mepacrine, azathioprine, lamprene, cyclophosphamide, a variety of sun-blocking creams and, more recently, Tigason. We are advised by the manufacturers of thalidomide that the use of this drug would be inadvisable because of its high incidence of severe side-effects, although Knop *et al.* (1983) reported success in treating 60 cases of discoid lupus erythematosus, with few side-effects.

### REFERENCE

KNOP, J., BONSMANN, G., HAPPLE, R., LUDOLPH, A., MATZ, D.R., MIFSUD, E.J. & MACHER, E. (1983) Thalidomide in the treatment of sixty cases of chronic discoid lupus erythematosus. *British Journal of Dermatology*, 108, 461.

## (13) Lupus erythematosus profundus

DR A.E.WALKER

*Chesterfield and North Derbyshire Royal Hospital*

B.N., female, aged 60 years.

*History.* In June 1974, this patient presented with alopecia and a rash on the nose and right ear lobe of 6 weeks duration. A diagnosis of discoid LE was made and confirmed histologically, and controlled subsequently with Dermovate® ointment and Spectraban® lotion. In April 1982, she re-attended with a 9-week history of increasing swelling of the left side of the face.

*Examination.* Tumid erythematous swelling of the left side of the face from the zygoma to the jawline and active discoid L.E. of the scalp were present.

*Investigations.* Haemoglobin and ESR are normal, but the white count is low at $3{\cdot}2 \times 10^9/\text{l}$. ANF and d/s DNA are negative.

*Histology.* Biopsy of the right side of the neck in 1974 showed atrophic epidermis, keratotic plugging and a lymphocytic infiltrate in the dermis, mainly around the adnexal structures. Biopsy of the left cheek in 1982 showed the epidermis to be essentially normal, but a dense lymphocytic infiltrate in the subcutaneous tissue.

*Treatment.* The eruption is controlled with prednisolone 5 mg per day and, in the last year, chloroquine 200 mg daily.

*Comment.* The left cheek now shows deep scarring. Active discoid L.E. is still present on the scalp.

### REFERENCE

FOUNTAIN, R.B. (1968) Lupus profundus. *British Journal of Dermatology*, **80**, 571.

## (14) Sub-acute cutaneous lupus erythematosus and malabsorption

DRS A.G.MESSENGER AND R.E.CHURCH

L.H., female, aged 40 years.

*History*. For 5–6 years this patient has complained of intermittent cramping abdominal pains with the passage of frequent bulky stools. This was investigated 3 years ago and a diagnosis of irritable bowel syndrome was made. Over the last 3 years, she has also complained of pain in the neck and right shoulder which has been treated with partial success with carbemazepine. In July 1985, a rash appeared on her face, neck and arms. Since then she has lost about 7 kg in weight.

*Examination*. There were raised red papules and plaques on the face, upper trunk and arms. Some lesions were annular with a ring of scale just inside the margin (Figs 1 and 2).

*Histology*. A skin biopsy showed mild hyperkeratosis and follicular plugging. The epidermis was thinned and there was marked degeneration of the basal layer. A lymphocytic infiltrate was present in the upper dermis, and around skin appendages. Direct immunofluorescence examination revealed granular deposits of C3, IgM and fibrin along the dermo-epidermal junction.

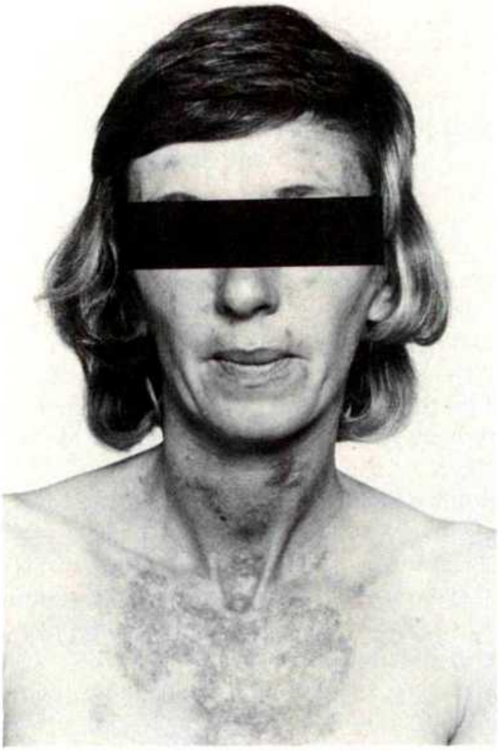

FIGURE 1. Papular and plaque lesions of lupus erythematosus on the face and anterior chest.

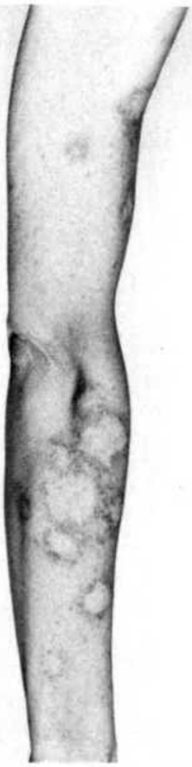

FIGURE 2. Annular lesions of lupus erythematosus on the forearm.

*Investigations*. Full blood count and ESR were normal, but the blood film showed a macrocytosis. Serum vitamin B12 (69 ng/l), folate (1·8 μg/l) and red cell folate (79 μg/l) were low. DNA antibodies were present in moderate titre (1 : 160), but other anti-nuclear antibodies were not detected. The serum C4 was marginally depressed (0·19 g/l). A 3-day faecal fat estimation showed marked elevation of fatty acid excretion (69·7 mmol/day, normal < 21 mmol/day). The following investigations were normal: biochemical screen, urinalysis, serum immunoglobulins, chest X-ray and jejunal biopsy.

*Comment*. The clinical and pathological features are those of sub-acute cutaneous lupus erythematosus (Sontheimer, Thomas & Gilliam, 1979). These patients are more likely to have systemic disease than those with discoid LE, although this is usually mild. The association of systemic LE with carbemazepine has been recorded, but withdrawal of the drug in this patient has not influenced the disease. She also has malabsorption and this may be the explanation for her long-standing, gastro-intestinal symptoms. Malabsorption has been reported in systemic LE (Siurala *et al*., 1965), but appears to be very uncommon. The cause of malabsorption in this patient has yet to be established and is currently being investigated. Treatment with chloroquine 400 mg/day and prednisolone 30 mg/day has so far proved ineffective.

## REFERENCES

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SONTHEIMER, R.D., THOMAS, J.R. & GILLIAM, J.N., (1979) Sub-acute cutaneous lupus erythematosus. A cutaneous marker for a distinct lupus erythematosus subset. *Archives of Dermatology*, **115**, 1409.

## (15) Protein losing enteropathy due to SLE

DRS M.L.WOOD AND R.E.CHURCH

B.W., female, aged 33 years.

*History*. This patient presented in 1982 with a 1-year history of intermittent periorbital oedema and more recent ankle oedema. She also complained of general malaise and lassitude. She had previously been investigated at another hospital for intermittent episodes of polyarthralgia, although no definite diagnosis had been made. She has secondary infertility of about 10 years standing, and Raynaud's phenomenon.

*Examination*. Apart from intermittent periorbital oedema, examination is unremarkable.

*Investigations*. Full blood count, serum calcium, phosphate and electrolytes, liver function tests, fasting blood glucose, creatine phosphokinase, serum B12, folate, iron and iron-binding capacity, X-rays of chest and hands, ECG, barium swallow, rectal biopsy, faecal fat excretion, IgG and IgA were all normal.

However, there was gross hypoalbuminaemia, 14 g/l (normal 35–53 g/l), total protein 42 g/l (normal 60–83 g/l), although 24 h urine estimations showed no excess of protein. ESR was raised at 114 mm/h, (normal 0–7 mm/h), blood urea 2·2 mmol/l (normal 3·3–8·5 mmol/l) and serum cholesterol 10·0 mmol/l (normal 3·8–7·0 mmol/l). Serum T4 and T3 were both low at 51 mmol/l (normal 60–170 mmol/l) and 0·5 mmol/l (normal 1·2–3·0 mmol/l), respectively, with a normal TSH.

Jejunal biopsy demonstrated normal villous architecture, although many villi appeared 'bloated', probably due to oedema. There was no evidence of Whipple's disease on light or

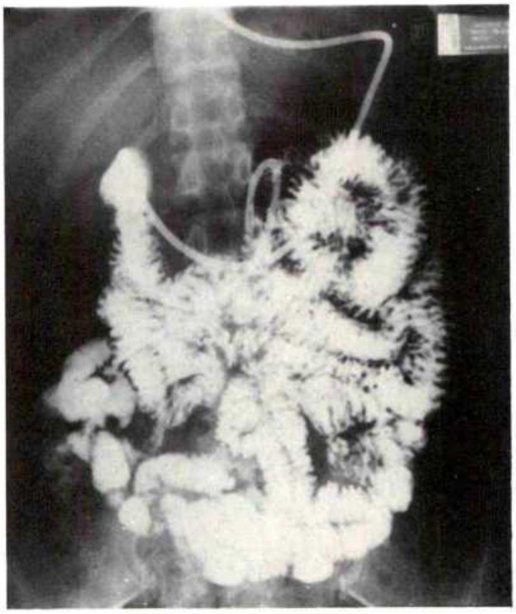

FIGURE 1. Small bowel enema showing a prominent mucosal pattern consistent with oedema.

electron microscopy. Barium meal and small bowel enema showed a prominent mucosal pattern consistent with oedema (Fig. 1).

An isotopic chromic chloride faecal excretion test was grossly elevated at 13·8% (normal 1·8%), indicating heavy protein loss from the bowel. Serum anti-nuclear antibodies were repeatedly detected in high titre (1:300) with a speckled pattern of staining using rat liver substrate, and a coursely granular pattern using Hep2 cells. ENA was present at 1:256, with specificity to RNP. Antibodies to DNA were not detected on numerous occasions, although plasma C3 and C3PA concentrations were repeatedly low, 0·5 g/l (normal 0·6–1·5 g/l) and 0·09 g/l (normal 0·5–0·45 g/l), respectively, with marked plasma C3 conversion (30%). Plasma C4 varied between 0·18–0·42 (normal 0·25–0·75 g/l). Serum immune complexes containing IgG and IgM were detected on several occasions, serum IgM being raised at 3·5 g/l (normal 0·5–2·0 g/l). Rheumatoid factor was present at 1:1280. There were no cryoglobulins or C-reactive protein. Light microscopy and direct immunofluorescence of normal exposed skin was unremarkable.

*Treatment.* Within a few days of commencing oral prednisolone, 60 mg daily, her facial oedema was much reduced, and within 3 weeks serum albumin had risen to 35 g/l, total protein to 65 g/l and ESR had fallen to 13 mm/h. A repeat faecal chromic chloride excretion test was within the normal range at 1·7%. Apart from intermittent episodes of lethargy, and one episode of depression treated with nomifensine, her condition has remained stable on prednisolone 15 mg daily with ACTH 20 units i.m. weekly. Attempts to reduce the prednisolone further have met with exacerbation of periorbital oedema, lethargy, arthralgia and depression.

*Comment.* It is now well known that certain subsets of SLE exist which do not demonstrate anti-nuclear DNA antibodies. This patient has none of the sclerodermatous features suggestive of mixed connective tissue disease, but satisfies the A.R.A. criteria for SLE and fits the group, previously well documented, of patients with SLE who possess anti-RNP antibodies but no anti-nuclear DNA antibodies. It is of interest that this particular group of SLE patients characteristically has a very low incidence of renal disease and hence an excellent prognosis. There have been very few cases reported of protein-losing enteropathy due to SLE, but in the one other case in which serum anti-nuclear DNA antibodies were measured, as in this patient, none were detected.

This case previously reported (Wood, Foulds & French, 1984), emphasizes the fact that chronic or periodic oedema should be investigated with a view to an underlying connective tissue disease.

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## (16) Lichen amyloidosis

Drs S.E.Thomas and R.E.Church

M.H., female, aged 51 years.

*History.* For many years, this patient had noticed areas of discoloration of the skin overlying the elbows and knees, and across the shoulders. From June 1985, the skin in these areas had

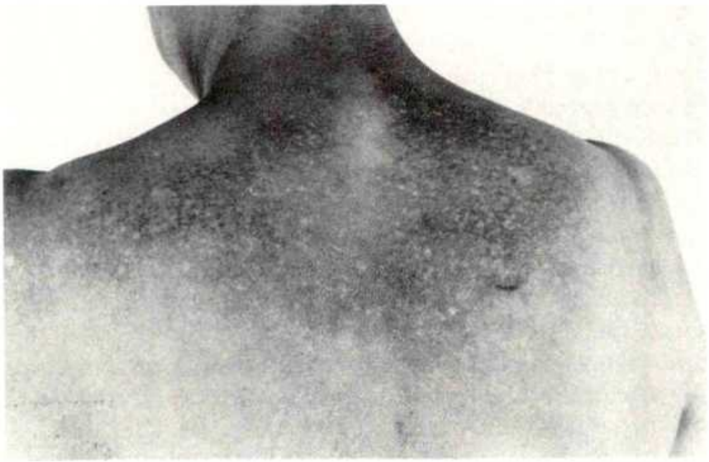

FIGURE 1. Pigmented plaques of lichen amyloidosis on upper back.

become 'thickened', but was never itchy. She had been investigated for haematuria in July 1983; all these investigations were negative, including cytoscopy, renal angiogram, abdominal ultrasound and EMSUs for acid-fast bacilli.

*Examination*. Extensive pigmented thickened plaques were noted, involving the extensor aspects of the elbows and knees, the buttocks and upper thighs and across the shoulders (Fig. 1).

*Investigations*. Haemoglobin, full blood count, immunoglobulins and IVP were all normal. The ESR was 59 mm/h.

*Histology*. The epidermis was normal. There is slight pigmentary incontinence with prominent melanophages within the dermis. The upper collagen appears scarred with an accentuation of collagen and contains a sparse perivascular lympho-histiocytic infiltrate. There is no evidence of pinching of adnexal structures. Amyloid is demonstrable by Thioflavin T fluorescence in the upper dermis. Immunohistochemistry showed no evidence of amyloid A or P component. Kappa and lambda light chains are not detected. Stains for cytokeratin were difficult to interpret due to non-specific uptake in the upper dermis. Electron microscopy demonstrates civatte body formation with collections of amyloid-like material in the upper dermis, some areas of which contain degenerate tonofilaments.

*Comment*. It has been proposed that the amyloid in lichen amyloidosis is derived from keratin. Yasu, Hosokawa and Seiji (1981) reported that antikeratin antiserum reacted with the amyloid in lichen amyloidosis. Noren *et al*. (1983) were unable to confirm this. So far, treatment for this condition is unsatisfactory.

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## (17) Primary amyloidosis

DRS S.B.BITTINER AND C.I.HARRINGTON

D.A., female, aged 54 years.

*History*. The patient has a 2-year history of blood blisters and erosions forming on the hands, following slight trauma. The lesions heal quickly leaving little scarring. She has also noticed a tendancy to bruise easily on the hands, and to a lesser extent on the arms, legs and face. She has occasionally developed erosions on her lower limbs after trauma. Her general health is good, and she feels well. She has had no serious illnesses.

*Examination*. There is purpura on the palmar and dorsal surface of the hands. The dorsal surfaces of both hands (Fig. 1) have erosions intermittently. Intact blisters have not been observed by medical staff. The nails are ridged longitudinally and there is oncholysis. Milia are present on the finger webs. Small areas of purpura are usually present on the legs, and sometimes on the trunk, face and buccal mucosa. The tongue is normal in size.

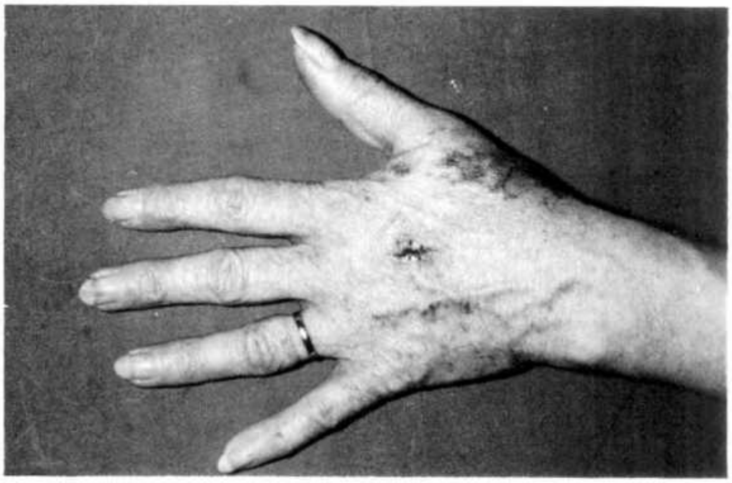

FIGURE 1. Purpuric lesions which appeared on the dorsum of the hand after minor trauma.

*Investigations*. Dip-stick testing of the urine on presentation showed 4+ protein, later quantified as 2·9 gm/day. The blood count shows a thrombocytosis of 565 × $10^9$/l. The urea, electrolytes, creatine clearance, and thyroid function tests are otherwise normal. The plasma immunoglobulins are normal, without a monoclonal band, and the urine is negative for Bence Jones protein. Bone marrow examination shows 12% polyclonal plasma cells. The other cell lines are normal. Complement studies are normal, and the ANF, anti-mitochondrial antibody, anti-smooth muscle antibody, and rheumatoid factor are negative. The porphyrins are not increased in the erythrocytes, and are absent in the urine and faeces. The chest X-ray shows cardiac enlargement but the ECG is normal. The urinary tract is normal on intravenous urography.

*Histology*. Light microscopy of the skin shows a hyaline eosinophilic material deposited principally in the dermal papillae, which stains positively for amyloid, and shows apple-green dichroism under polarized light. There is a cleavage between this material and the adjacent dermis, with the true dermo-epidermal junction intact. Direct immunofluorescence of the skin is negative. Immunological stains for amyloid proteins show large amounts of confluent globular IgG beneath the basement membrane zone with scattered areas of similar staining in the dermis. Amyloid P protein, but not amyloid A protein, is present in a similar distribution. In addition, there is some homogeneous IgG and amyloid P staining of the blood vessels in the dermis. Electron microscopy confirms amyloid deposition. The renal biopsy shows mild peripheral loop thickening and mesangial expansion by eosinophilic material. The arteries and arterioles are significantly thickened, and infiltrated by the eosinophilic material, which is positive for sirius red stains and is dichromic in polarized light. The material is resistant to potassium permanganate oxidation suggesting AL type protein amyloid.

*Comment.* This patient's renal function remains good despite the renal amyloid, so no drug treatment has been commenced. A combination of melphalan with prednisone has been shown to reduce protein excretion in patients with primary amyloidosis affecting the kidney, although survival was not influenced (Kyle & Greipp, 1978). Treatment with DMSO (Ravid *et al.*, 1982) and colchicine (Scheinberg, Pernambuco & Benson, 1984) have proved disappointing in this condition.

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## (18) Systemic mastocytosis

Drs W.F.G.Tucker and C.I.Harrington

J.P., male, aged 70 years.

*History.* This patient presented in 1980 with a 40-year history of diarrhoea and a skin rash. Twenty years previously, 'colitis' had been diagnosed on sigmoidoscopy. The severity of his diarrhoea varied, with up to 20 bowel actions daily. During exacerbations, the rash was noticeably more evident and hot flushes occurred. Maturity onset diabetes mellitus was diagnosed in 1970 and controlled with dietary changes and chlorpropamide, the latter occasionally resulting in confusing 'alcohol' hot flushes.

*Examination.* Examination reveals a widespread rash affecting the trunk and limbs consistent with the telangiectasia macularis perstans variant of urticaria pigmentosa. The liver is palpable three fingerbreadths below the costal margin.

*Investigations.* 'Normal' investigations included haemoglobin, full blood count, serum B12 and folate, faecal fats, liver function tests, urea and electrolytes, thyroid function, 5 hydroxy-indole acetic acid excretion, a skeletal survey, bone scan and (in 1985) aspiration of bone marrow.

Skin biopsies show the typical appearance of urticaria pigmentosa.

A barium meal in 1980 showed a small sliding hiatus hernia, and coarse gastric rugae, indicating hyperacidity. In the duodenum and small bowel, many nodular space-filling defects

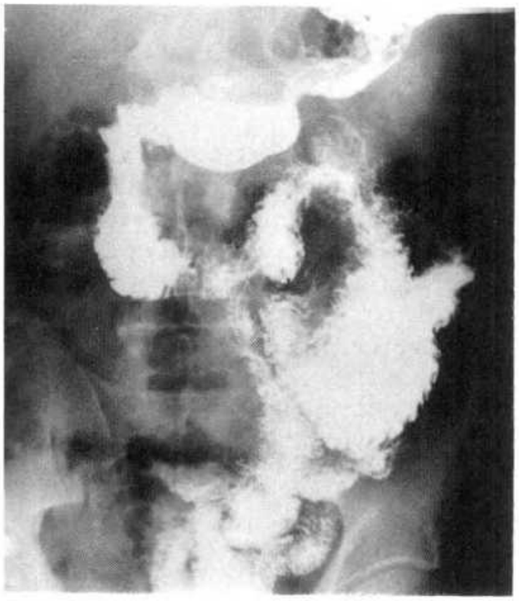

FIGURE 1. Barium follow-through examination showing nodular space-filling or 'honeycomb' defects in the small bowel, characteristic of intestinal mastocytosis.

were evident on follow-through examination (Fig. 1). Endoscopy confirmed a mild gastritis. The rectal mucosa was oedematous and both rectal and jejunal biopsies revealed evidence of mast cell infiltration. Trephine bone marrow examination (1985) shows numerous clumps of cells with the staining properties of mast cells. A lymph-node biopsy in 1985 showed normal nodal architecture, but scattered mast cells around the perivascular regions. An isotope scan of the liver and spleen showed a patchy uptake of tracer in the liver, and a relatively reduced uptake in the spleen.

*Treatment*. Disodium cromoglycate was started in November 1980, and the dose increased to 100 mg q.d.s. A gradual decrease in the frequency of bowel activity was noted over the next few months, until after one year's treatment the present level of control (3 actions daily) was achieved. Codeine phosphate is still occasionally required. The flushing attacks also ceased, possibly due to concomitant alcohol abstinence.

*Discussion*. The duration of this man's gastro-intestinal symptoms, which persisted essentially unchanged for 40 years, is unusual in systemic mastocytosis. Although presumably he has had systemic disease for this period, we have no evidence of transformation from the benign state. The radiological appearances appear to be fairly specific for mast-cell involvement of the gut.

Oral disodium cromoglycate has successfully controlled our patient's symptoms for 5 years, with minimal inconvenience.

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SOTER, N.A., AUSTEN, K.F. & WASSERMAN, S.I. (1979) Oral disodium cromoglycate in the treatment of systemic mastocytosis. *New England Journal of Medicine*, **301**, 465.

## (19) Systemic mastocytosis

DRS W.F.G.TUCKER AND C.I.HARRINGTON

D.G., female, age 72 years.

*History*. In 1976, while recovering in hospital from an acute haemorrhage from a duodenal ulcer, this patient was noted to have scattered lesions of urticaria pigmentosa on her legs. As far as she could recall, the symptomless brown marks had been present for 2 years. Following an uneventful vagotomy and pyloroplasty, she was not seen again in the skin clinic until re-referred in September 1985, suffering from acute pompholyx.

Over the intervening 9 years, the original rash had become progressively more extensive. Her only symptoms remain occasional episodes of itching in hot weather, a 'burning' sensation of the skin and watery diarrhoea approximately once a month.

*Examination*. A very extensive urticaria pigmentosa rash is present, with brown maculo-papules and plaques over the limbs and trunk and extending to involve the face and neck (Fig. 1). There was no detectable hepatosplenomegaly.

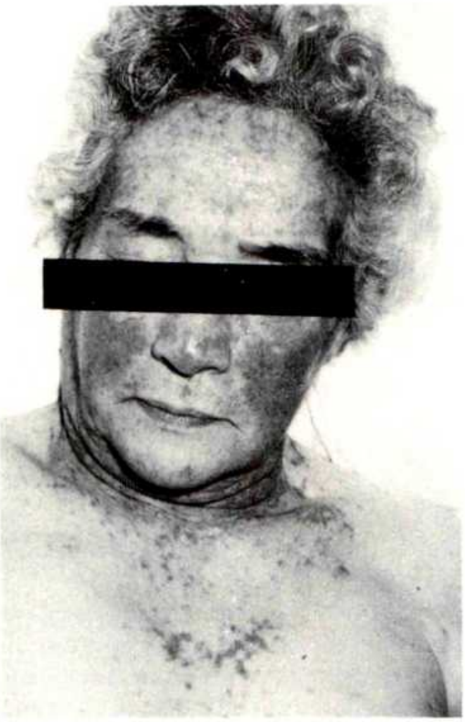

FIGURE 1. Maculo-papular eruption of mastocytosis on the face and neck.

*Investigations*. Skin biopsies showed epidermal acanthosis, scarring of the papillary dermis with dilated blood vessels and a heavy infiltrate of mast cells, typical of mastocytosis. Normal investigations included routine biochemistry, plasma cortisol, ECG, chest X-ray and skeletal survey. A barium enema showed only diverticular disease. Her haemoglobin was 14·4 g/dl.,

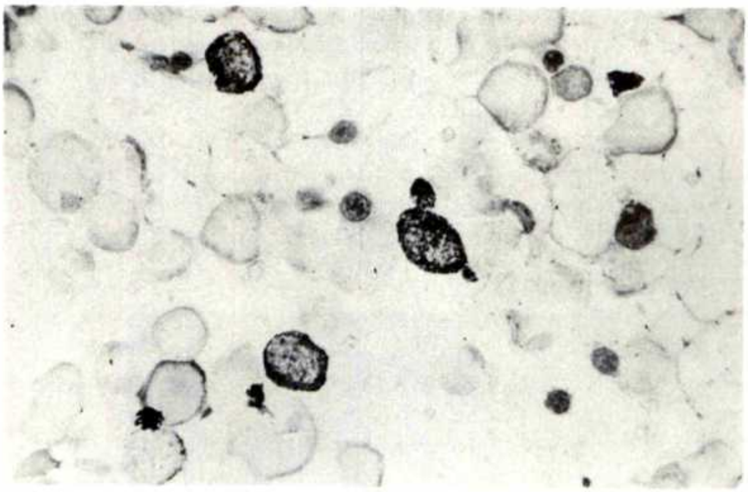

FIGURE 2. Bone marrow aspiration showing an increased mast cell population (toluidine blue stain).

white count 11·8 × $10^9$/l, platelets 346 × $10^9$/l and ESR 39 mm/h. The differential count showed a slight neutrophilia (8·14 × $10^9$/l) and a slight eosinophilia (0·47 × $10^9$/l). Aspiration bone marrow examination (Fig. 2) showed an increase in the mast cell and basophil/eosinophil series consistent with marrow involvement by systemic mastocytosis.

The histology of a groin lymph node revealed expansion of the interfollicular and sinusoidal areas by a mixed infiltrate of eosinophils, mast cells and histiocytes, with focal deposition of melanin. These features are those of dermatopathic reactivity with skin mastocytosis.

*Treatment*. At present, our patient has insufficient symptoms to warrant starting her on either antihistamines or disodium cromoglycate. If her itching becomes more troublesome or her appearance begins to cause distress, we may use PUVA.

*Comment*. Although this lady clearly has systemic mastocytosis, with infiltration of her bone marrow by abnormal mast cells, she still has active, normal haemopoietic precursors. She is also relatively untroubled by her disease and we therefore plan to withold cytotoxic therapy until we have definite evidence of 'overflow' into the blood. An additional factor encouraging us in our non-interventionist management is the absence of an effective chemotherapeutic regime for this rare disorder.

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## (20) Reticular erythematous mucinosis (R.E.M. syndrome)

DR R.E.CHURCH

C.H., female, aged 38 years.

*History*. This schoolteacher first developed a rash on her chest and back at the age of 6 years.

This fluctuated and itched when hot, especially in summer. At the age of 23, years the rash became worse and more persistent. At first, it improved on treatment with local corticosteroids, but by 1975 had become constant. A biopsy at that time was consistent with the diagnosis of reticular erythematous mucinosis syndrome.

Since 1980, she has been treated with chloroquine starting with 200 mg twice daily which cleared the rash in 12 weeks. The dose has been reduced gradually to 200 mg once weekly which keeps the skin clear; if the chloroquine is stopped the eruption recurs after a month or two.

*Examination.* There are infiltrated, reticular and erythematous areas on the chest and upper back (Fig. 1).

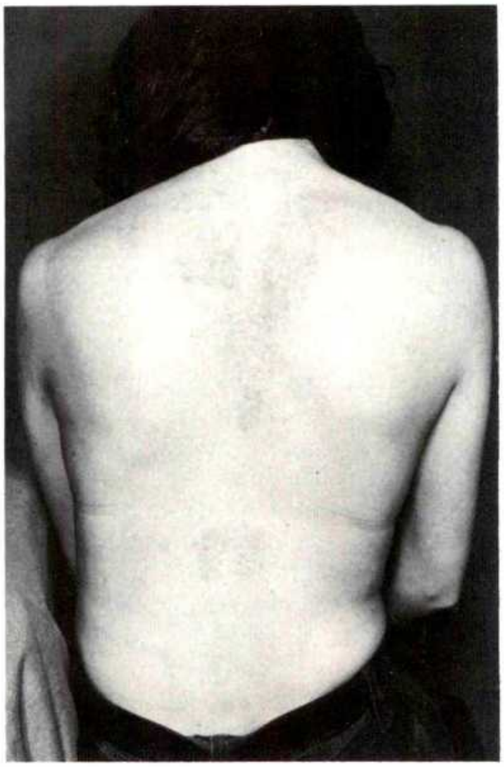

FIGURE 1. V-shaped infiltrated, reticular, erythematous area on the upper back.

*Investigations.* Skin biopsy showed a dermal mononuclear cell infiltrate (composed largely of lymphocytes), separation of collagen bundles and fragmentation of elastic fibres. Histochemical stains showed increased dermal mucin with a profile consistent with hyaluronic acid. Light testing was negative for UVA, and showed a response to approximately 3 MED of UVB which produced a persistent erythema at the test site. Antinuclear factor was negative.

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## (21) Darier's disease

A.G.Messenger for R.E.Church

A.A., female, aged 34 years.

*History*. During the summer months for the past 3 years, this patient has developed a pruritic rash on the forehead and trunk. This improves during the winter, but does not clear completely. Her son and daughter have a similar rash.

*Examination*. There are sheets of reddish-brown scaly papules on the upper trunk, around the neck and on the temples. The nails show longitudinal ridging with V-shaped nicks at the free edge. Her two children are similarly affected.

*Histology*. There is follicular hyperkeratosis with grains and corp ronds. Suprabasal clefts are present and there is focal epidermal hyperplasia. The features are those of Darier's disease.

*Treatment*. Topical treatment with 0·1% retinoic acid has produced considerable improvement.

## (22) Erythrokeratoderma variabilis (Mendes da Costa's syndrome)

Drs W.F.G.Tucker and C.I.Harrington

A.L., female, aged 44 years.

*Examination*. When first seen in our clinic, extensive plaques of erythema and hyperkeratosis

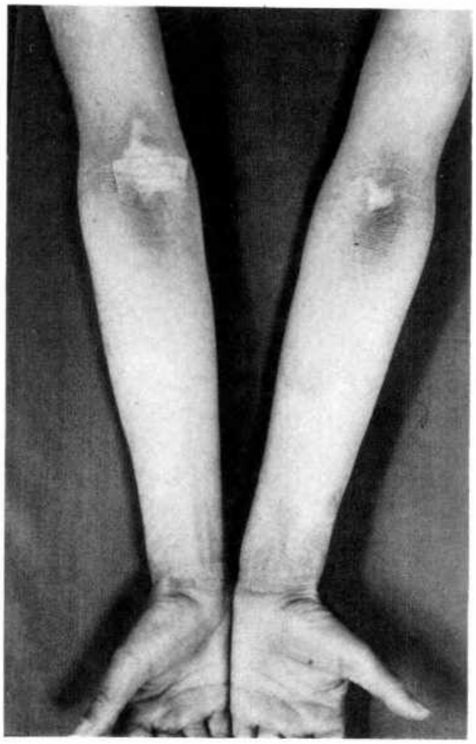

FIGURE 1. Plaques of erythema and hyperkeratosis on the forearms.

were present on the upper chest and back, the flexor surfaces of the arms (Fig. 1), the knees, the popliteal fossae and the feet.

*Investigations.* The liver function tests and her lipid profile have remained normal.

*Histology.* A biopsy performed in Manchester was consistent with erythrokeratoderma variabilis.

*Treatment.* Etretinate was started in July 1981 and there was a marked improvement within 2 weeks. Currently, she is controlled on 30 mg daily.

## (23) Erythrokeratoderma variabilis (Mendes da Costa's syndrome)

DRS W.F.G.TUCKER AND C.I.HARRINGTON

P.L., male, aged 15 years.

*History.* This young man's skin eruption first appeared at the age of 8 months, starting on the hands and spreading to the trunk, limbs and chin. The rash is aggravated by sun exposure, and tends to clear in the winter. The patient's mother, A.L., has the same condition.

*Examination.* Plaques of erythema and hyperkeratosis were present on the trunk and limbs at presentation.

*Investigations.* Liver function tests and his lipid profile have remained normal.

*Treatment.* Vitamin A 50000 units daily was commenced in May 1974, resulting in a worthwhile improvement in his condition. In particular, he noticed that he was less sun-sensitive. Etretinate was started in June 1982 when he was aged 13 years. Within 3 weeks he was completely clear. Currently, he is taking 20 mg/10 mg etretinate on alternate days.

*Comment.* The quality of this patient's life, and that of his mother, has been transformed by the use of etretinate, serving to remind us what a genuine therapeutic advance the development of retinoids has been.

## (24) Lymphomatoid papulosis and poikiloderma

DRS S.B.BITTINER AND R.E.CHURCH

J.H., male, aged 53 years.

*History.* The patient presented in 1972 with papules on the right wrist and subsequently on the face, limbs and trunk. In 1977, he developed poikiloderma of his sacrum, and then on his arms. In 1979, an infiltrated plaque appeared on his elbow.

*Examination.* There are scattered papules, and a few areas of poikiloderma.

*Investigations.* The full blood count, ESR, urea, electrolytes, liver function tests and chest X-ray are normal. Toxoplasma and Brucella screens are negative.

*Histology.* Sections of the skin show a dense lymphohistiocytic infiltrate in the dermis with a predominantly peri-vascular and peri-adnexal distribution. Atypical lymphocytes and much larger atypical cells are present in the infiltrate. The infiltrate extends into the vascular walls where there is endothelial swelling and focal extra-vasation of erythrocytes.

*Treatment.* The patient was treated with topical steroids, but the condition remained active, especially on unexposed areas. He commenced PUVA in 1978. He manages to keep the disease activity suppressed with one treatment per week.

*Comment.* Follow-up studies of patients with lymphomatoid papulosis have found malignant change in 0–20% of patients (Lange-Wantzin, Thomsen & Hou-Jensen, 1984; Sanchez *et al.*, 1983, respectively). It is not clear whether treatment alters the susceptibility to malignant change.

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## (25) Pityriasis lichenoides developing into mycosis fungoides

DRS S.S.BLEEHEN AND D.N.SLATER

S.B., male, aged 30 years.

*History.* Since the age of 2 years, the patient has had typical lesions of pityriasis lichenoides. At the age of 9 years, areas of poikiloderma were noted. These have extended over the years to involve his neck, back, thighs and buttocks. Marked hair loss has developed in the neck and occipital region of the scalp where pustular and cystic lesions have occurred.

In 1979, he was referred from Rotherham District General Hospital for PUVA treatment. His response to PUVA was good, but the areas of poikiloderma have extended and milia-like lesions have continued to occur. In May 1985, he developed an ulcerated tumour on the back of the right thigh (Fig. 1). Enlarged inguinal lymph nodes were present at that time. The tumour and surrounding infiltrated area was treated with topical nitrogen mustard with improvement. His photochemotherapy has been continued.

*Examination.* There are extensive areas of poikiloderma involving face, neck, trunk and limbs. A residual indurated area is present on the back of the right thigh with superficial ulceration of the skin. Several infiltrated scaly macules are present on the trunk and limbs. On the face, neck, back and particularly in the occipital region of the scalp there are many milia/pustular cysts associated with hair loss. In both groins there are enlarged lymph nodes. The liver and spleen are not enlarged.

*Investigations.* The full blood count, ESR, bone marrow and chest X-ray were all normal. No Sézary cells were seen in the peripheral blood. The immunoglobulins were normal apart from a raised IgE ( > 1000 IU/ml). There was marked depression of cellular immunity and the response of lymphocytes to phytohaemagglutinin was markedly depressed. Analysis of the chromosomes of lymphocytes showed non-clonal abnormalities.

*Skin biopsy.* A dense dermal lymphoid infiltrate composed of hyperchromatic small and larger 'blast' forms was present. Some epidermotropism of these cells with micro-abscess formation was noted. The infiltrate consisted almost entirely of Leu3 and Leu4 positive cells. In the dermis there were many OKT6 positive (Langerhans) cells.

*Treatment.* In October 1985, he was re-admitted for a further course of topical nitrogen mustard. His photochemotherapy with oral psoralens has been continued and there has been further improvement.

*Comment.* This patient has evolved from having typical pityriasis lichenoides lesions as a child to then developing extensive areas of poikiloderma, and now has mycosis fungoides. There is histological evidence of lymph node involvement.

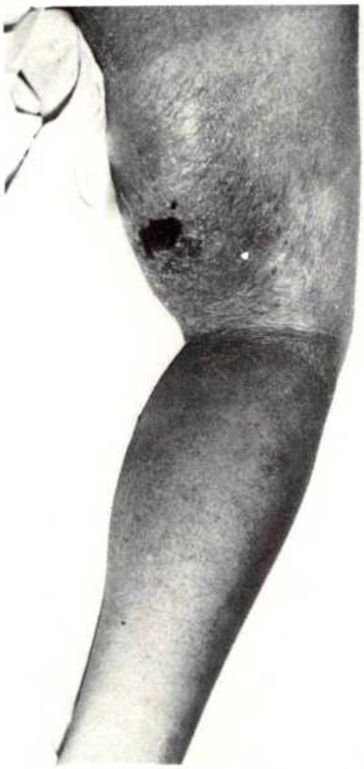

FIGURE 1. Ulcerated indurated area on back of right thigh.

The association of pityriasis lichenoides with poikiloderma and evolving into mycosis fungoides is well recognized (Samman, 1971). This patient has had typical lesions of pityriasis lichenoides and other cyst and milia-like lesions such as occur in the related lymphomatoid papulosis (Black, 1982). Detailed histological studies on lymph node biopsies from this and other patients with mycosis fungoides (Slater *et al.*, 1985) have shown that so-called 'dermatopathic lymphadenitis' is not benign, and there is often involvement with convoluted lymphocytes that are helper T cells.

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## (26) Pre-reticulotic eruption with alopecia

Drs D.M.Lister and R.A.C.Graham-Brown

*Leicester Royal Infirmary*

P.C., female, aged 66 years.

*History and examination.* The patient first developed a dry, red, itchy skin in 1960. In 1967, she was referred to St John's Hospital for Diseases of the Skin, by which time she had a generalized, erythematous, scaly rash sparing only her palms, soles and parts of her face. A diagnosis of chronic eczema was made, but further biopsies were recommended. By 1981, when seen at the Royal Free Hospital, she had developed almost total alopecia with some areas of erythema and scale around the mouth of each residual hair follicle (Fig. 1). Her cutaneous eruption remained generalized and had become more pigmented, with, in some areas, a lichenoid appearance. A degree of epidermal atrophy was also apparent, especially over the flanks. She has now been transferred to the care of the Dermatology Department, Leicester Royal Infirmary. Her skin has become increasingly pigmented and poikilodermatous. The alopecia persists and is now static.

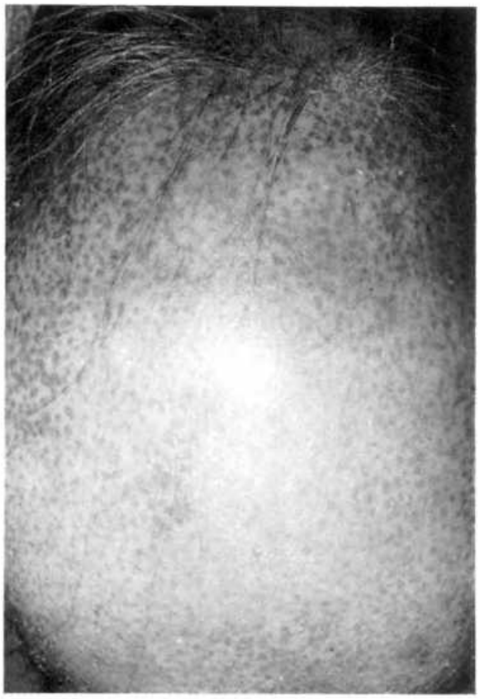

FIGURE 1. The scalp shows almost complete hair loss. Remaining follicles are accentuated by perifollicular scale and erythema.

*Investigations:* (a) *histology*. Biopsies in 1967 showed a perivascular lymphocytic infiltrate in the upper dermis with some patchy spongiosis and parakeratosis of the epidermis. This was provisionally labelled as eczema, but Professor Wilson Jones expressed concern about this and advised histological follow-up. Further histological specimens were obtained in 1981 and 1983 from abdominal skin and scalp. These showed collections of lymphohistiocytic cells in the papillary dermis with a slight tendency to epidermal invasion. Some of the infiltrating cells were large and hyperchromatic, suggestive of Sézary-Lutzner cells. The scalp biopsies showed, in general, a less-dense infiltrate but a marked reduction in follicle numbers. There was no evidence of follicular mucinosis. The most recent biopsy (1985) shows a heavy cellular infiltrate in an upper dermal band. There are a number of cells with hyperchromatic and convoluted nuclei, and the infiltrate extends focally into the overlying epidermis. The appearance is that of a pre-reticulotic eruption.

(b) *Cell marker studies*. These confirm that the infiltrate is largely composed of T cells, the helper/suppressor ratio being 9:1.

*Treatment*. The patient has been treated over the years with emollients, topical steroids and a course of systemic steroids. More recently, she has been given a course of PUVA without obvious change.

*Comment*. This lady illustrates clearly that pre-reticulotic eruptions may begin very insiduously and that continued follow-up and repeat biopsies are essential. A most unusual feature, however, is the development of an extensive cicatrical alopecia. There is no mention of this possible complication of early stage mycosis fungoides in the major texts.

## (27) Lymphomatoid granulomatosis

DRS M.L.WOOD AND C.I.HARRINGTON

J.E., male, aged 35 years.

*History*. Over the past $8\frac{1}{2}$ years, this patient has had several tender, subcutaneous lumps of varying size. Three of these were excised successfully from the anterior abdominal wall, left axilla and right shoulder, and one resolved spontaneously. The other two, over the left sacro-iliac area and left flank, became extensively ulcerated (Fig. 1). This has been associated with

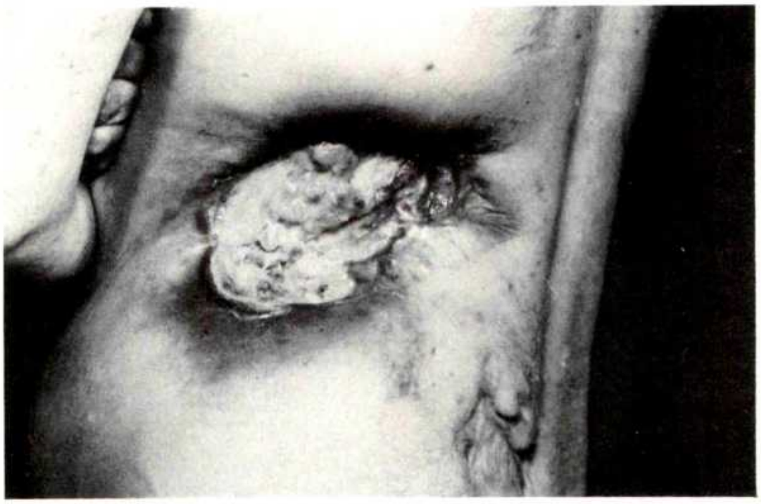

FIGURE 1. Large ulcer of lymphomatoid granulomatosis in the left flank and scarring from a previous cutaneous lesion.

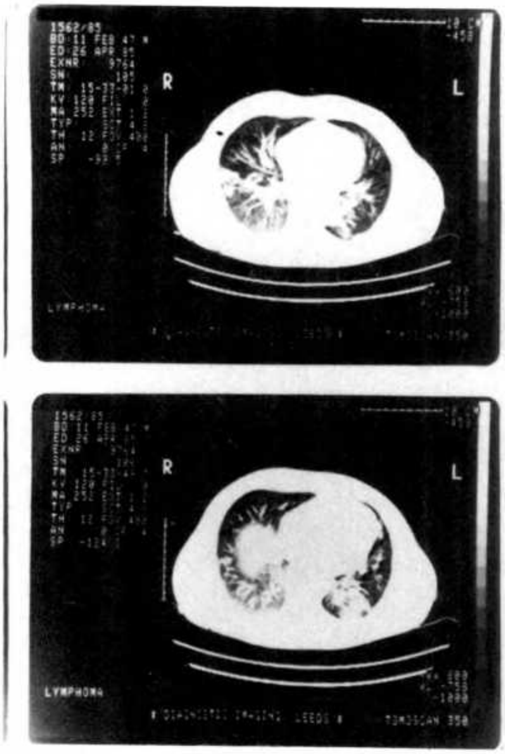

FIGURE 2. CAT scan showing multiple bilateral pulmonary infiltrates.

intermittent pyrexia, general malaise, mild pleuritic chest pain and dyspnoea, and intermittent lower abdominal pain and dysuria. In the past, he had a detached retina in 1961 and an episode of asthma in 1970. There is no relevant family history.

*Examination*. He has extensive scarring in the left lumbar and sacral areas, and scars from surgical excision of previous lesions. He shows early clubbing of the finger nails, but his chest is clinically clear and there is no hepatosplenomegaly or lymphadenopathy.

*Investigations*. Throughout his illness he has had an intermittent microcytic hypochromic anaemia and lymphopaenia, with a raised ESR, and initial non-specific elevation of liver enzymes and immunoglobulins. Chest X-ray shows patchy bilateral infiltrates in mid and lower zones. Respiratory function tests show a minimal reduction in transfer factor at 7·0 (predicted 8·4–11·2). Total body CAT scan also shows extensive bilateral pulmonary infiltrates (Fig. 2). Culture of sputum, skin and liver for *Mycobacterium tuberculosis* was negative, and previous liver biopsy, abdominal lymphangiogram and bone marrow trephine were normal. Peripheral blood shows an absolute lymphopaenia ($< 0{\cdot}5 \times 10^9/l$), with a disproprotionately low T-cell count (44% sheep erythrocyte rosetting) and a high T4 (helper/inducer) to T8 (suppressor/cytotoxic) cell ratio of 4·4:1, resulting from a low number of peripheral T suppressor cells. In addition, a very high proportion (19%) of peripheral blood lymphocytes show a marker for immature cells (T10). No monoclonal immunoglobulins or free light chains have been detected in blood or urine.

*Histology*. Skin biopsies from lesions in the left flank and anterior abdominal wall both showed extensive necrosis of subcutaneous fat and a dense mixed cellular infiltrate, predomin-

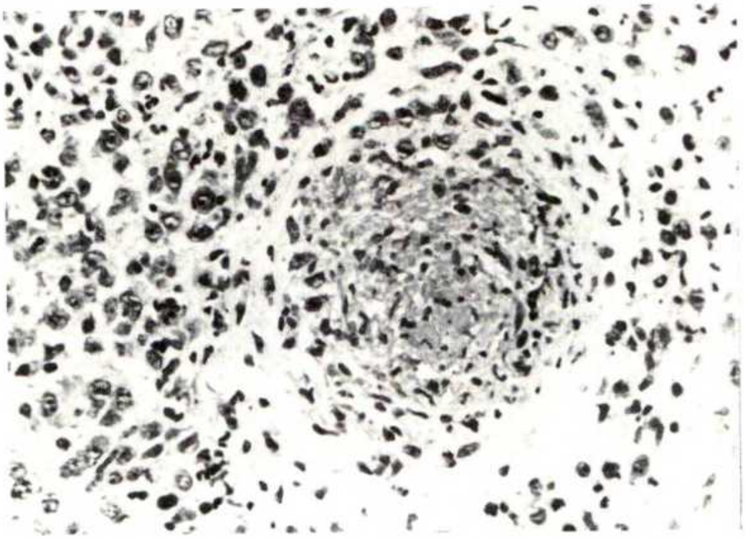

FIGURE 3. Skin biopsy from anterior abdominal wall (original × 800 approximately). Angiocentric and angiodestructive infiltrate with large numbers of atypical cells.

antly lymphocytes with small groups of plasmacytoid cells, polymorphs and occasional large atypical mononuclear cells with hyperchromatic nuclei (Fig. 3). Multinucleated cells were infrequent and Reed-Sternberg cells absent. The infiltrate extended into the lower dermis, surrounding eccrine sweat glands and small nerve fibres, and involving the walls of small and large veins and arteries. There was no caseation and stains for AFB were negative. Monoclonal antibody immunoperoxidase studies revealed large numbers of T4 (helper/inducer) lymphocytes with a few T8 (suppressor/cytotoxic) lymphocytes, and T10 (immature lymphocyte) positive cells were also present. Staining for lysozyme and $\alpha 1$ anti-trypsin showed moderate numbers of histiocytes, and the scattered plasma cells present showed only IgA lambda positivity.

*Treatment.* The large ulcer in his left flank finally healed after $2\frac{1}{2}$ years, during which time he has had several courses of 'high dose' intravenous cyclophosphamide (1 g i.v. 3 weekly) and alternate day prednisolone 10/20 mg, local radiotherapy (1450 rads on a linear accelerator), surgical debridement and plastic repair. He is at present taking prednisolone 10 mg on alternate days.

*Comment.* Lymphomatoid granulomatosis was first described by Liebow, Carrington and Friedman (1972). Histologically, it is characterized by an angio-centric and angio-destructive lymphohistiocytic proliferation. The infiltrate is polymorphous with granulomatous features of Wegener's granulomatosis with the cellular atypicality of lymphoma. Progression of the disease in some cases of frank lymphoma is well documented, but may possibly be prevented by early treatment with a combination of prednisolone and cyclophosphamide, or radiotherapy (Fauci *et al.*, 1982).

Although this patient still shows obvious pulmonary involvement, he remains relatively asymptomatic. In view of the presence of plasma cells showing monoclonal lambda light chain production, however, it may be only a matter of time before a B-cell malignant lymphoma develops. This case was reported in the *B.J.D.* (Wood *et al.*, 1984).

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WOOD, M.L., HARRINGTON, C.I., SLATER, D.N., ROONEY, N. & CLARK, A. (1984) Cutaneous lymphomatoid granulomatosis: a rare case of cutaneous ulceration. *British Journal of Dermatology*, **110**, 619.

## (28) Cutaneous sarcoidosis

DRS S.E.THOMAS AND R.E.CHURCH

D.S., female, aged 79 years.

*History*. This elderly patient developed scaly red lesions on the forehead in 1983. She is otherwise asymptomatic.

*Examination*. Scaly erythematous, granulomatous, well-demarcated lesions were noted on the forehead (Fig. 1). Clinical examination was otherwise normal.

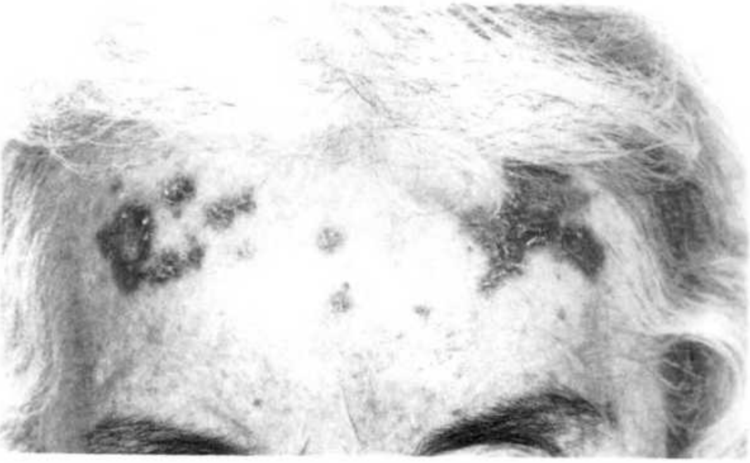

FIGURE 1. Well-demarcated granulomatous lesions on the forehead.

*Investigations*. Haemoglobin, full blood count, ESR, respiratory function tests, VDRL, serum calcium and 24-h urinary calcium were all normal. Serum angiotensin converting enzyme was 63 U/ml/min (normal less than 52 U/ml/min). The chest X-ray showed slight cardiac enlargement, the Kveim test was negative, and the Mantoux test 1:1000 was negative.

*Histology*. The epidermis appears normal. Numerous epithelioid granulomata containing giant cells are seen within the dermis with loss of elastic fibres. Many of the giant cells contain asteroid bodies. Stains for acid/alcohol fast bacilli and fungi are negative.

*Treatment*. In view of this patient's age, it has been decided merely to cover these lesions with covermark.

*Comment*. There is no evidence of systemic involvement in this patient. The lesions are located in the light-exposed areas on the forehead. O'Brien (1985) describes necrobiotic and sarcoid variants of actinic granuloma. He suggests that the lesions start with actinic elastosis and ultimately manifest as cutaneous sarcoidosis.

### REFERENCE

O'Brien, J.P. (1985) Actinic granuloma: the expanding significance. An analysis of its origin in elastotic ('aging') skin and a definition of necrobiotic (vascular) histiocytic and sarcoid variants. *International Journal of Dermatology*, **24**, 473.

## (29) Sarcoidosis

Drs S.E.Thomas and C.I.Harrington

B.T., male, aged 41 years.

*History*. In 1976, the patient developed lesions on the face and one buttock at the site of a previous scar. In 1968, he had attended the chest clinic in Stockport, where pulmonary sarcoid was diagnosed. In 1977, a skin biopsy from a facial lesion revealed cutaneous sarcoid. He has subsequently been treated with topical steroids, chloroquine, izoniazid and systemic steroids.

*Examination*. Multiple scaley, granulomatous, scarring lesions were present over both cheeks, the nose (Fig. 1), the buttocks and the knees. He had a hoarse voice, but clinical examination was otherwise normal.

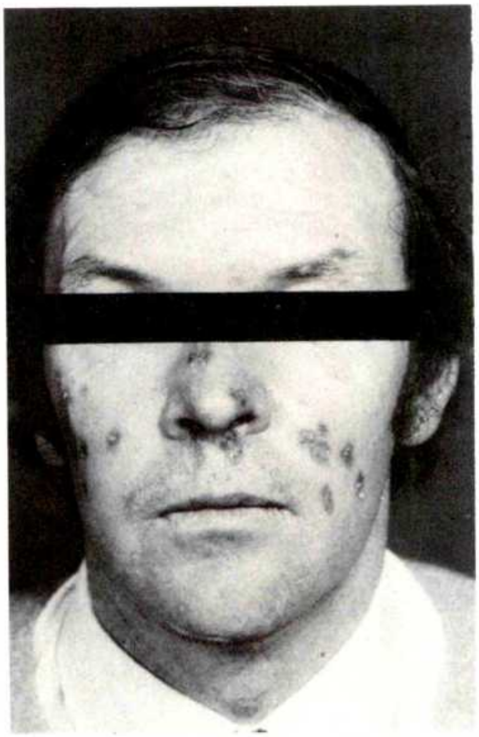

FIGURE 1. Disfiguring, scarring facial lesions of sarcoidosis.

*Investigations.* Liver biopsy, full blood count, serum calcium and 24-h urine for calcium were normal. Angiotensin-converting enzyme was 53 U/ml/min (normal less than 52 U/ml/min). Respiratory function tests were as follows: spirometry normal and transfer factor 10·2 mmol/min. Chest X-ray showed bilateral hilar lymphadenopathy and a basal interstitial pattern. Liver biopsy was normal, Kveim test was positive, liver function tests showed $\gamma$-glutamyl transferase 243 U/l (normal 2–45 U/l) and SGPT 65 U/l (normal 7–45 U/l).

*Histology.* The skin biopsy shows giant and epithelioid cell non-caseating tubercules, consistent with sarcoid. Stains for fungi and acid-alcohol fast organisms are negative. The lung biopsy shows compressed lung parenchyma with a thickened small pulmonary artery surrounded by fibrotic alveoli in which there are two epithelioid granulomata, consistent with sarcoidosis.

*Treatment.* In February 1984, the patient received prednisolone 60 mg daily for 3 weeks. He is maintained on prednisolone 5 mg daily.

*Comment.* In sarcoidosis, treatment is indicated for destructive lung disease, uveitis, hypercalcaemia, and disfiguring skin lesions. It is uncertain whether systemic steroids actually alter the natural course of the disease (Sharma, Colp & Williams, 1966). In steroid-resistant cases, methotrexate may be effective in cutaneous sarcoidosis (Veien & Brodthagen, 1977).

### REFERENCES

SHARMA, O.P., COLP, C. & WILLIAMS, M.H. (1966) Course of pulmonary sarcoidosis, with and without corticosteroid therapy as determined by pulmonary function studies. *American Journal of Medicine*, **41**, 541.

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## (30) Sarcoidosis

DRS A.G.MESSENGER AND S.S.BLEEHEN

P.G., female, aged 32 years.

*History.* The patient is a negro from the West Indies. Two years ago she noticed an area of pale skin on the left forearm. Subsequently, further pale patches have appeared on the face, arms and upper thighs. Her general health is good and there is no relevant past medical or family history.

*Examination.* There are several poorly defined infiltrated hypopigmented plaques on the face, arms and upper thighs. Sensation within the lesions is intact. General examination is normal.

*Histology.* A biopsy from a hypopigmented lesion revealed numerous dermal granulomata in the vicinity of adnexal structures. A few were close to nerves. No foreign material or micro-organisms were identified.

*Investigations.* The following investigations were normal: full blood count, ESR, urea and electrolytes and serum calcium. A chest X-ray showed bilateral hilar lymphadenopathy. The lung fields were clear. Respiratory function tests revealed a moderate reduction in transfer factor. A Kveim test was strongly positive.

*Comment.* Hypopigmented plaques are a recognized feature of cutaneous sarcoidosis in West Indian and American negroes (Mayock *et al.*, 1963; Clayton *et al.*, 1977).

### REFERENCES

CLAYTON, R., BREATHNACH, A., MARTIN, B. & FEIWEL, M. (1977) Hypopigmented sarcoidosis in the negro. Report of eight cases with ultrastructural observations. *British Journal of Dermatology*, **96**, 119.

Mayock, R.I., Bertrand, P., Morrison, C.E. & Scott, J.H. (1963) Manifestations of sarcoidosis. *American Journal of Medicine*, **35**, 67.

## (31) Granulomatous cheilitis

Drs S.E.Thomas and S.S.Bleehen

*Children's Hospital, Sheffield*

C.D., female, aged 13 years.

*History*. In 1977, this girl presented in Liverpool with recurrent swelling of the upper lip. Her family subsequently moved to Sheffield, and in 1981 she presented to the Dermatology Department with persistent swelling of the lower lip. She had been treated unsuccessfully with prolonged courses of antibiotics. She has had no other symptoms. In October 1985, she developed a perianal abscess. She suffered from infantile eczema as a baby. There is no family history of relevance.

*Examination*. The lower lip is chronically swollen (Fig. 1) and the buccal mucosa is thickened and corrugated in appearance. The tongue is normal. She has a prominent anal skin tag and a healed perianal abscess.

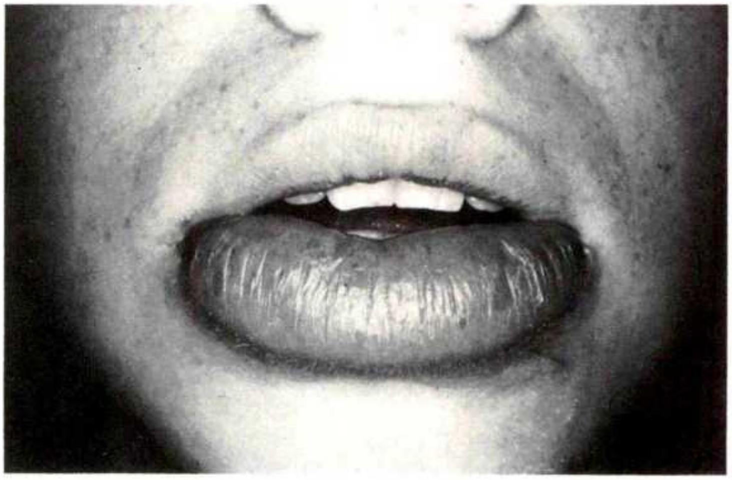

FIGURE 1. Chronically swollen lower lip of granulomatous cheilitis.

*Investigations*. Haemoglobin, full blood count, ESR, liver function tests, serum B12, folate and angiotensin converting enzyme were all normal.

*Histology:* (a) *skin biopsy* (lip). Dermal granulomas are seen in the lip raising the possibility of Crohn's disease.

(b) *Perianal lesion*. A granuloma with histiocytes and epithelioid cells is present in the wall of the perianal abscess cavity. This appearance would be compatible with Crohn's disease.

*Treatment*. The treatment so far has included long-term antibiotics and elimination diets, without any success. Local injections of triamcinolone into the lip result in improvement for a few months at a time. More recently, she has been receiving 5-amino-salicylic acid 500 mg twice daily. She is currently being investigated by a paediatric gastro-enterologist.

*Comment*. Granulomatous cheilitis is a rare but recognized early manifestation of Crohn's disease (Carr, 1974). The cheilitis may precede other gastro-intestinal manifestations by several years, as in this case (Brook, King & Miller, 1983). Patton *et al.* (1985) have attempted to treat patients with oro-facial granulomatosis using elimination diets, with limited success. Our patients presented 8 years ago with cheilitis and now appears to be developing other signs of Crohn's disease.

## REFERENCES

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CARR, D. (1974) Granulomatous cheilitis in Crohn's disease. *British Medical Journal*, **ii,** 656.

PATTON, D.W., FERGUSON, M.M., FORSYTH A. & JAMES, J. (1985) Oro-facial granulomatosis: A possible allergic basis. *British Journal of Oral and Maxillofacial Surgery*, **23,** 235.

## (32) Cutaneous Crohn's disease

DRS S.E.THOMAS AND R.E.CHURCH

M.H., female, aged 46 years.

*History*. This patient presented in May 1974 with multiple perianal and vulval skin tags. A biopsy of a skin tag revealed Crohn's disease. In July 1974, a panproctocolectomy and ileostomy

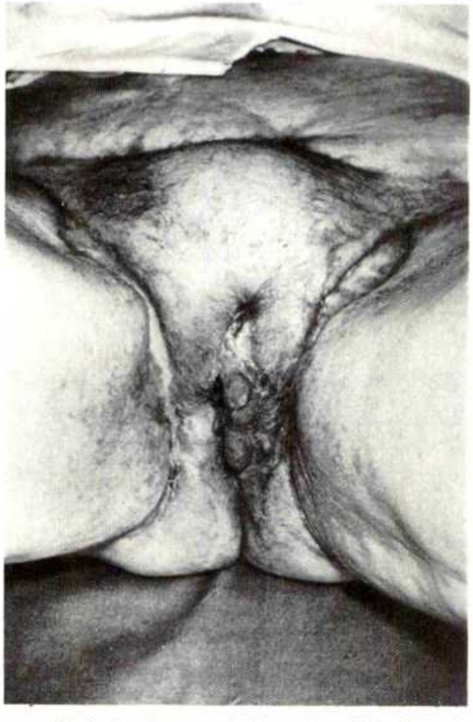

FIGURE 1. Vulval oedema and ulceration of Crohn's disease.

was performed. The entire large bowel was affected by Crohn's disease. Although there was no recurrence of gastro-intestinal Crohn's disease, the patient still had perineal fistulae and vulval oedema. In 1977, she received oral penicillin for 3 months, and in 1978, she was treated with azathioprine 100 mg daily for 6 months. These measures produced little change. By July 1980, she had developed deep fissures in the natal cleft, groins and lower abdominal skin folds. Injections of triamcinolone were unhelpful.

*Examination.* On admission to hospital in December 1983, she had severe intertrigo and deep fissures in the groins and lower abdomen, and gross perineal oedema (Fig. 1).

*Investigations.* Serum ferritin, serum B12 and folate, urea, electrolytes, liver function tests and plasma proteins were all normal. In July 1980 and February 1984, the small bowel barium meal and follow-through were normal. In July 1983, haemoglobin was 11·2 g/dl, white blood count $6{\cdot}0 \times 10^9$/l, platelets $278 \times 10^9$/l and ESR 60 mm/h, serum iron 5 $\mu$mol/l (normal 9–32), total iron binding capacity 56 $\mu$mol/l (normal 45–75). Skin swabs grew mixed staphylococci, but no candida.

*Histology.* A biopsy from a fissured abdominal skin fold revealed granulomas consistent with Crohn's disease.

*Treatment.* This patient is receiving prednisolone 10 mg daily and azathioprine 50 mg daily, and is well controlled.

*Comment.* This is a typical case of cutaneous Crohn's disease (Mountain, 1970). There is no consistent relationship between skin lesions and bowel symptoms. Treatment of the gastro-intestinal disease has cleared some, but not all, patients of their disease.

### REFERENCE

MOUNTAIN, J.C. (1970) Cutaneous ulceration in Crohn's disease. *Gut*, **11**, 18.

## (33) Crohn's disease

DRS S.E.THOMAS, R.E.CHURCH AND C.D.HOLDSWORTH

R.E., male, aged 53 years.

*History.* In 1969, this patient was investigated for abdominal pain, weight loss and occasional rectal blood loss. Sigmoidoscopy revealed a chronic anal fissure and a sentinal pile. In 1975, he started developing nodules and ulceration on the lower legs. In 1981, he developed intermittent bloody diarrhoea, and ultimately an ischio-rectal abscess. In 1984, he underwent a laparotomy for resection of the terminal ileum, and the diagnosis of Crohn's disease was established. He also suffers from psoriasis, angina and diabetes mellitus.

*Examination.* On examination, the psoriasis is currently involving the limbs. Intermittently, pyoderma gangrenosum is present, affecting the anterior shins.

*Investigations.* Haemoglobin was 13·4 g/dl, white blood count $8{\cdot}9 \times 10^9$/l, platelets $381 \times 10^9$/l, erythrocyte sedimentation rate 28 mm/h, and blood glucose 5·6 mmol/l. Liver function tests and urea and electrolytes were all normal. Autoantibodies were negative.

*Histology.* The skin biopsy (1975) has been reviewed. It shows an active vasculitis with a granulomatous lobular panniculitis. When this is considered with the histology of the small bowel, a diagnosis of cutaneous Crohn's disease is a strong possibility.

The section of the small bowel (1984) shows active ileitis with fissuring, ulceration and abscess formation. Lymphoid aggregates show transmural penetration with small collections on the serosal surface. There is perforation, giving rise to serosal inflammation. These appearances are consistent with Crohn's disease.

*Treatment.* The patient is currently receiving prednisolone 10 mg daily, glyceryl trinitrate as required, and propranolol 80 mg three times daily.

*Comment.* This patient presented with vague gastro-intestinal symptoms 15 years before a firm diagnosis of Crohn's disease was made. In retrospect, his skin lesions in 1975 were those of cutaneous Crohn's disease, and these also occurred 9 years before Crohn's disease was confirmed. Metastatic cutaneous Crohn's disease had been reported previously (Lebwohl *et al.*, 1984; Witkowski, Parish & Lewis, 1977), although it is rare, and all patients have involvement of the colon or rectum.

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WITKOWSKI, S.A., PARISH, L.C & LEWIS, J.E. (1977) Crohn's disease—non-caseating granulomas on the legs. *Acta Dermato-Venereologica*, **57**, 181.

## (34) Atrophic morphoea

DRS. W.F.G.TUCKER AND R.E.CHURCH

R.M., female, aged 24 years.

*History*. At the age of 14, this patient first noticed large brown patches developing on her back. Morphoea was diagnosed at Rotherham District Hospital and she was not seen again until she came to the clinic in 1983, having developed new smaller lesions over the past year, which were slightly pruritic.

*Examination.* Two large brown atrophic plaques are present in the centre of the back. The texture and appearance of the overlying epidermis is normal.

*Investigations.* Auto-antibody screen is negative.

*Histology.* A skin biopsy shows only minor changes, with no evidence of sclerosis.

*Comment.* Our patient's sex, the age of onset, the morphology of her lesions and their unremarkable histology, correspond closely to the cases of progressive idiopathic atrophoderma described by Pasini and Pierini (Canizares *et al.*, 1958), but which most dermatologists now consider to be an atrophic variant of morphoea.

REFERENCE

CANIZARES, O., SACHS, P.M., JAIMOVICH, L. & TORRES, V.M. (1958) Idiopathic atrophoderma of Pasini and Pierini *Archives of Dermatology*, 77, 42.

## (35) Diabetes, necrobiosis lipoidica and granuloma annulare

DRS M.L.WOOD, S.S.BLEEHEN AND J.D.WARD

H.C., female, aged 27 years.

*History*. This patient, with insulin-dependent diabetes since the age of 8, first noticed lumpy areas within the skin on the feet and left wrist 16 years ago. They remained asymptomatic and unresponsive to topical fluorinated steroids until 7 years ago when she developed new lesions over the shins, with atrophy and intermittent ulceration of some of the old lesions. There is no family history of diabetes or skin disease. Medication consists of Neusulin 12 and Neulente 34 units each morning.

*Examination.* She has typical areas of necrobiosis lipoidica, intermittently ulcerated, over the lower legs, and lesions more typical of granuloma annulare on the left forearm and feet. There is mild background diabetic retinopathy.

*Investigations.* Random blood glucose is 15·6 mmol/l. Glycosylated haemoglobin is raised at 81 mmol HMF/mol Hb (29–39 mmol HMF/mol Hb normal range). Skin biopsy taken from the left foot shows the edge of a palisading granuloma with necrotic collagen and fragmented elastic fibres. The appearances are consistent with either granuloma annulare or necrobiosis lipoidica.

*Treatment.* There has been no response to Dermovate® ointment under polythene occlusion or soluble aspirin 300 mg bd orally for 14 months. However, some clinical improvement has occurred in areas of necrobiosis injected with triamcinolone.

*Comment.* Granuloma annulare-like lesions may occur in patients with necrobiosis lipoidica (Muller & Winkelmann, 1966). Not infrequently, as in this case, they can evolve into typical lesions of necrobiosis lipoidica. Atypical and more generalized forms of granuloma annulare are frequently associated with diabetes mellitus and as in necrobiosis lipoidica there is evidence of a micro-angiopathy.

### REFERENCE

MULLER, S.A. & WINKELMANN, R.K. (1966) Necrobiosis lipoidica diabeticorum. Histopathologic study of 98 cases. *Archives of Dermatology*, **94**, 1.

## (36) Granuloma annulare

DRS W.F.G. TUCKER AND S.S.BLEEHEN

E.G., female, aged 58 years.

*History.* This patient presented in December 1983 with a 10-month history of a non-itchy rash affecting the left hand and both arms. Since then the lesions have increased in size and new ones have appeared on both forearms and legs.

*Examination.* Initially, she had annular purplish plaques on the dorsum of the left hand and in both cubital fossae. These have increased in extent over the past 2 years.

*Investigations.* A glucose tolerance test was normal. No abnormality was seen on the chest X-ray.

*Skin biopsy.* This showed typical features of granuloma annulare with foci of collagen degeneration in the dermis surrounded by mononuclear cells and occasional giant cells.

*Treatment.* She was treated with clobetasol propionate and with flurandrenolone-impregnated tape, and although there was improvement, treatment had to be stopped because of skin atrophy. Intralesional injections of triamcinolone were given into the edges of the more active lesions.

*Comment.* The progression of the disorder in this patient suggests that she has a more persistent type of granuloma annulare that will become more generalized (Wells & Smith, 1963). There is in this type of more generalized granuloma annulare an increased incidence of diabetes mellitus, although this was not manifest in our patient.

REFERENCE

WELLS, R.S. & SMITH, M.A. (1963) Natural history of granuloma annulare. *British Journal of Dermatology*, 75, 199.

## (37) Benign familial pemphigus (Hailey-Hailey disease)

DRS W.F.G.TUCKER AND S.S.BLEEHEN

M.G., male, aged 41 years.

*History*. The patient had a 15-year history of recurrent itchy lesions with occasional blisters on the neck and trunk. These were more frequent in hot humid weather. His mother was also affected.

*Examination*. Annular erythematous lesions with vesiculation at the margins were present on the sides of the neck and on the central part of the back. The axillae and groins were unaffected.

*Skin biopsy*. There was clefting of the basal portion of the epidermis with marked acantholysis. In the upper dermis there was a mild perivascular inflammatory infiltrate. Direct immunofluorescence studies of the skin biopsy were negative.

*Comment*. The unusual location of the lesions on this patient's back was explained by his occupation as a bus driver. Since his employer has provided him with a cloth-covered seat on his bus, he has found that the frequency of exacerbations during the summer has been greatly reduced.

## (38) Parasitophobia and linear IgA disease

DRS S.E.THOMAS AND R.E.CHURCH

J.S., male, aged 56 years.

*History*. This patient presented in 1978 with recurrent blisters affecting the groin, wrists, buttocks and feet (Fig. 1). He was treated with dapsone 100 mg daily, but relapsed in 1979. He

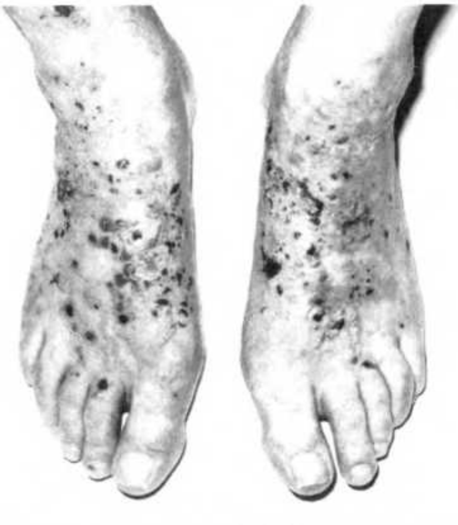

FIGURE 1. Large, tense bullae on the feet when the patient presented in 1978.

then received dapsone 150 mg daily plus prednisolone 40 mg daily, and a gluten-free diet. His skin remained moderately well controlled until 1981, when he developed parasitophobia after his grandson had had thread worms. His condition has relapsed and remitted subsequently, despite treatment with sulphapyridine, dapsone, prednisolone and pimozide.

*Examination.* Widespread, grouped, excoriated lesions affecting the trunk, scalp and limbs were present. Clinical examination was otherwise normal.

*Investigations.* Haemoglobin, full blood count, liver function tests, urea and electrolytes, serum complement, immunoglobulins, antibasement membrane antibody and antidesmosome antibody were all negative. A patch test to potassium iodide was negative.

*Histology.* Sub-epidermal bullae containing fibrin, numerous neutrophil and eosinophil polymorphs were present. Direct immunofluorescence demonstrated linear basement membrane staining for IgG and IgA in involved and uninvolved skin.

*Treatment.* The patient is currently receiving dapsone 200 mg daily and prednisolone 15 mg daily.

*Comment.* He remains convinced that this problem is caused by parasites.

## (39) Epidermolysis bullosa simplex. Mental and physical handicap

DR C.I.HARRINGTON

E.B., female, aged 6 years.

*History.* Four days after birth by caesarian section, this baby developed large haemorrhagic blisters, mainly on the hands and feet, but also affecting the trunk and limbs.

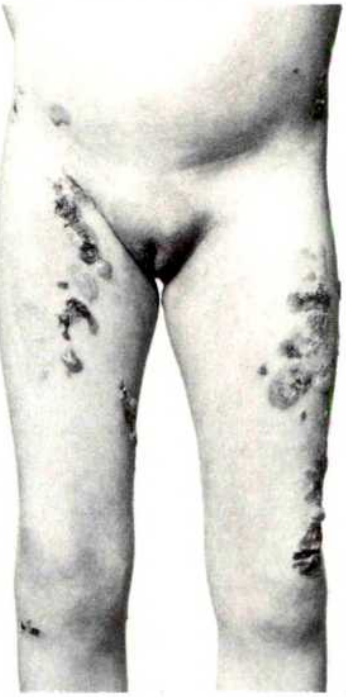

FIGURE 1. Tense haemorrhagic blisters on upper thighs.

*Family history.* There is no history of bullous disorders, but her father has a 'handicapped' child by a previous marriage, and suffers himself from neurofibromatosis. He also had two brothers who died of leukaemia.

*Examination.* During the first few weeks of life she had very large blisters, particularly on sites of friction. These ruptured to leave large eroded areas. Mucous membranes have not been affected. Blisters are now mainly confined to the hands and feet, upper arms and thighs (Fig. 1). She has a pigmented naevus on the left thigh.

*Histology.* PAS staining and electron microscopy have shown that the split is situated above the basal lamina. There is marked degeneration of the basal cells with 'amyloid-type' body formation. The hemidesmosomes, sub-basal dense plaques, anchoring filaments, lamina lucida, basal lamina and anchoring fibrils all appear normal.

*Treatment.* This child failed to thrive, despite hospitalization in the first 2 weeks of life. Prednisolone phosphate 20 mg daily produced dramatic improvement in the skin and general condition. This dose was gradually reduced, and after 6 months she was well controlled on prednisolone 2·5 mg on alternate days. At the age of 3 years, she was taken off steroid therapy and tests of adrenal function were normal.

*Comment.* Systemic steroid therapy played an important role in control of blister formation in this handicapped child's early years. Developmental enhancement programmes continue to improve her retarded development.

## (40) Two siblings with epidermolysis bullosa

DRS M.L.WOOD, R.E.CHURCH AND M.E.KESSELER

V.G., male, aged 13 years.
L.G., female, aged 6 weeks.

*History.* V.G. has had continual blistering and erosions of the skin with minimal trauma, since birth, resulting in extensive scarring and encasement of the hands and feet. At the age of 7 years, he had clearance of the first dentition for severe caries, but his permanent teeth are now relatively healthy. He has no dysphagia. In 1976 and in March 1984, he underwent plastic surgery to separate the fingers on both hands. There was no previous history of EB, but his mother has recently had another baby (female), who is also unfortunately affected, although the other sister is not.

*Examination.* V.G. shows extensive scarring and erosion of the skin, particularly over the pressure areas and extremities, with resultant 'mitten' deformity of the hands and feet. There are usually several scattered flaccid blisters. The other child L.G. shows a glove and sock distribution of haemorrhagic bullae with erosions, oral erosions and milia at birth. Truncal lesions have been rare.

*Histology.* Sections through an early blister in V.G. show an established sub-epidermal bulla associated with remodelling of the architecture of the papillary dermis, consistent with dystrophic EB. Electron microscopy also shows dermal scarring, but no diagnostic features. The infant's skin biopsy showed a sub-basal lamina cleft. The specimen was unsuitable for electron microscopy.

*Treatment.* Unfortunately, the older child has had no apparent benefit from oral phenytoin and zinc sulphate, given for at least 1 year. More recently, he was commenced on Tigason® 10 mg daily, but this was discontinued after 2 months as he was still developing frequent new blisters. L.G. has not received systemic therapy to date.

## (41) Recessive dystrophic epidermolysis bullosa

DRS M.L.WOOD AND C.I.HARRINGTON

M.L., female, aged 44 years.

*History.* This patient has had recurrent, widespread blistering and erosions of the skin since birth. She has noticed increasing dysphagia over recent years for which she has had numerous oesophageal dilations, but still depends largely on a liquidized diet. She also has bilateral conductive deafness for which she wears a hearing aid. Despite her problems, she has lived a reasonably full life, working as a telephonist until 1973. Unfortunately, repeated plastic surgical procedures on her hands, both as a child and over the past 3 years have been of little benefit.

In April 1985, she received laser therapy for cervical intra-epithelial neoplasia. Shortly afterwards she had a squamous cell carcinoma on the dorsum of the right hand excised and grafted. There is no relevant family history.

*Examination.* This patient shows extensive blistering and erosion of the skin, especially over the forearms, hands and legs, with scarring and characteristic 'mitten' deformity of the hands. She also has ectropion and wears a wig because of scarred alopecia.

*Histology.* Sections from involved skin of the left forearm show a sub-epidermal blister. Electron microscopy demonstrates microblister formation in a sub-basal lamina position, with sparsity of anchoring fibrils, but essentially normal collagen bundles.

*Treatment.* She has been taking prednisolone 10 mg daily for many years. The addition of phenytoin 100 mg daily for a period of about 18 months in 1980/81 resulted in little noticeable improvement. She takes vitamin C 1 g/day Abidec elixir, and uses Zincoderm® cream and Bactroban® topically.

## (42) Dominant dystrophic epidermolysis bullosa (Pasini variant)

DRS M.L.WOOD AND C.I.HARRINGTON

J.G., female, aged 40 years.

*History.* This patient has developed blistering on pressure areas since shortly after birth. In recent years, the elbows and anterior aspect of the lower legs have been the most troublesome regions. In addition, over the last 10–15 years he has noticed the appearance of 'white spots' on his forehead and upper back. He is otherwise well and there is no one else in the family similarly affected, including his twin sister.

*Examination.* This patient shows blistering and scaling predominantly over the anterior aspect of the lower legs and over the elbows, together with atrophic scars and milia. He also has small ivory-coloured papules scattered over the upper back and forehead (Fig. 1), together with nail dystrophy. There is hyperhidrosis of the feet. His mouth and teeth appear to be normal.

*Investigations.* Histological examination of sections of involved skin showed superficial epithelial ulceration with acanthotic and parakeratotic reactive changes. The superficial dermis shows granulation tissue and architectural reorganization, with destruction of elastic fibres down to the deep reticular dermis.

Electron microscopy shows that there are diminished numbers of anchoring fibrils with sub-epidermal bulla formation, but hemidesmosomes, anchoring filaments and lamina densa appear normal. Both light and electron microscopy of uninvolved skin are normal.

*Treatment.* There has been doubtful benefit in the past from phenytoin 300 mg daily. At

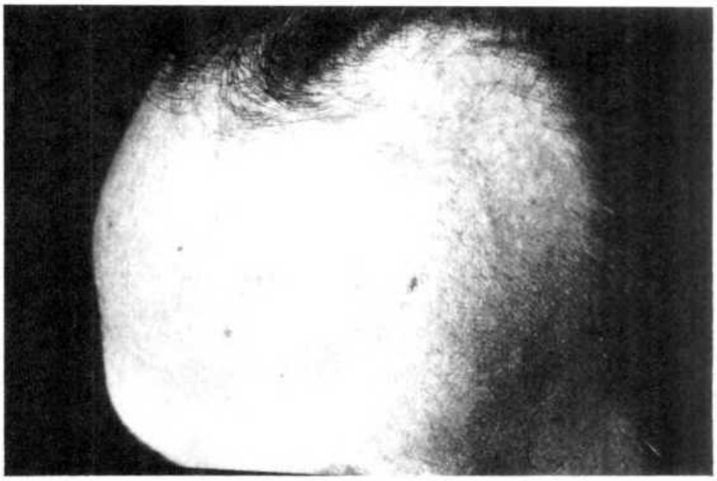

FIGURE 1. Albo-papuloid lesions on the forehead typical of this variant of epidermolysis bullosa.

present, itching of affected areas is reasonably controlled with Triludan 60 mg b.d. and he is using glutaraldehyde for the hyperhidrosis.

*Comment.* Clinically, this man is an example of the albo-papuloid or Pasini variant of dominant dystrophic epidermolysis bullosa. The blistering is said to be slightly more troublesome than the other variant, and the white papules are characteristic.

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## (43) Gunther's disease (congenital erythropoietic porphyria)

DR C.I.HARRINGTON

J.C., female, aged 30 years.

*History.* In 1955, it was noticed that this patient produced pink urine when she was 2 days old. This recurred, and porphyrins were demonstrated in the urine at the age of 4 months. Haemolytic anaemia developed when she was $5\frac{1}{2}$ months old, her haemoglobin dropping to 4·8 g/100 ml with 5% of red cells showing red fluorescence in ultraviolet light. Blood transfusion did not improve her anaemia, and splenectomy was performed in 1956. The large spleen, weighing 95 g, showed intense fluorescence under ultra-violet light.

Later in 1956, on her first sun exposure, she developed blisters on light-exposed areas. Her erupting teeth were brown and fluoresced in ultra-violet light. Scarring and hypertrichosis became marked on light-exposed areas throughout her early childhood. During this time, she required three or four blood transfusions a year for repeated haemolytic crises.

*Family history.* Her parents and four siblings are healthy.

*Examination*. During her life, progressive scarring with sclerodermatous thickening and hyperpigmentation has affected the skin of her face and hands. She has marked cicatricial alopecia. Her discoloured teeth have now been capped.

*Investigations*. There is constant evidence of haemolytic anaemia. Plasma, urinary, and faecal porphyrins have been grossly elevated throughout her life. In 1985, total urine porphyrins were 4500 mg/l, mainly uroporphyrin by thin layer chromatography (normal 20–210 mg/l). Total faecal porphyrins were 1000 mg/g dry weight ($>98\%$ coproporphyrin—normal $<150$ mg/g dry weight). Total plasma porphyrins remain greatly elevated at 717 mg/l (normal 3–12 mg/l), and total erythrocyte porphyrin is 665 mg/dl packed rbc (normal 0–95 mg/dl rbc). Erythrocyte uroporphyrinogen III synthetase 2·18 nmol/h/mg haemoglobin (reference range: 4·56–6·70 nmol/h/mg) is diagnostic for congenital erythropoietic porphyria.

*Histology*. A skin biopsy taken from the back of the left hand in 1973 showed that the dermis contained mucopolysacharide which extended into the dermal papillae, particularly around the blood vessels. Some areas of calcification were present in the dermis.

*Treatment*. All sunscreens used up to 1973 were totally ineffective and she was forced to lead the life of a recluse. In 1973, beta-carotene 25 mg t.d.s. was commenced and produced a marked improvement in her light tolerance.

Repeated blood transfusions for anaemia have led to iron overload and she now requires treatment for this with subcutaneous desferrioxamine.

## (44) Bullous eruption on haemodialysis (pseudoporphyria)

DRS M.L.WOOD AND C.I.HARRINGTON

D.L., male, aged 48 years.

*History*. This patient was first referred in September 1985 with a 2-year history of blisters on his hands. He complained that his skin was fragile and blisters resulted from minor injury. Sunlight did not appear to worsen the problem. He also complained of generalized itching.

He had suffered from chronic renal failure due to glomerulonephritis since 1961. Haemodialysis was commenced in 1975. Three renal transplants in 1977, 1979 and 1985 have failed. He is

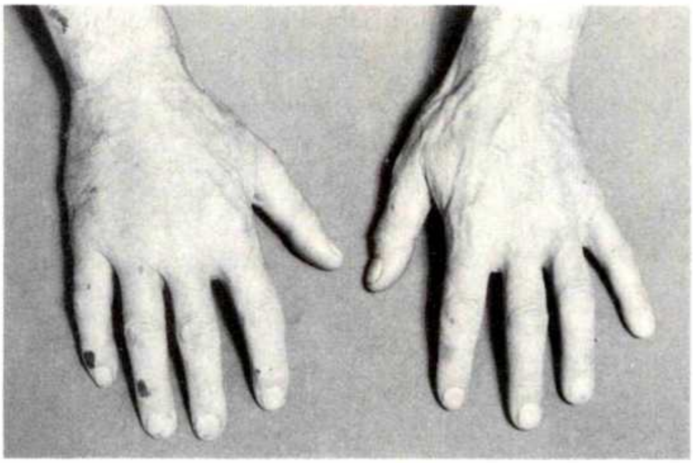

FIGURE 1. Erosions and haemorrhagic bullae on the dorsal surfaces of the hands.

currently haemodialysed three times a week. He has not taken any drugs known to cause bullous eruptions.

*Examination*. He has small intact blisters and erosions on the dorsal aspect of both hands (Fig. 1), the forehead and scalp.

*Investigations*. Urine and faecal porphyrins are not elevated. Total plasma porphyrin is 24 μg/l (normal 3–12 μg/l), but levels up to 48 μg/l are usual in chronic renal failure.

*Histology*. Skin biopsy from the right hand shows a sub-epidermal blister with a little chronic inflammation. There is also ulceration with associated acute inflammation and sclerosis of the upper dermis.

*Comment*. The slightly elevated concentration of plasma porphyrin is not regarded as high enough to produce bullous lesions due to photosensitization by porphyrins (G.Elder, personal communication). The cause of this bullous eruption of haemodialysis is therefore uncertain. It is interesting that the lesions cleared after his renal transplant in March 1985, but recurred a few weeks after rejection and recommencement of haemodialysis.

He gains some benefit from avoidance of trauma and sunlight and reduction of his alcohol consumption but changes in the permeability of his dialysis membrane do not influence his lesions.

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## (45) Persistent erythema multiforme

DRS S.B.BITTINER AND R.E.CHURCH

S.W., female, aged 38 years.

*History*. The patient has a 19-year history of recurrent mouth ulceration with bullous lesions on the lower legs. She feels off-colour and ill during the attacks. The condition became quiescent during her last pregnancy 2 years ago, but has since recurred.

*Examination*. Characteristic lesions of erythema multiforme occur on the lips, buccal mucosa and on the legs. There are palpable lymph nodes in the neck and left axilla.

*Investigations*. The full blood count shows a neutrophil leucocytosis during an attack. The last white cell count was 15·9 × 10⁹/l (neutrophil, 11·54 × 10⁹/l). The haemoglobin, platelet count, ESR, urea, electrolytes and liver function tests are normal. Viral swabs from the mouth have been negative. Serum immunoglobulins are normal, but IgM immune complexes have been present in each assay.

*Histology*. The section shows a sub-epidermal bulla. Focal necrotic keratinocytes are present within the roof and also within the adjacent epidermis. There is a perivascular infiltrate of lymphocytes and histiocytes. There are no significant DIF findings.

*Treatment*. Levamisole 200 mg twice weekly was used for 3 months, early in 1983. Later the same year, she had six injections of human normal immunoglobulin 640 mg intramuscularly. She was admitted in December 1983 for two units of fresh frozen plasma. None of these treatments produced a significant clinical response. This year she has had a 4-week course of Inosine Pranobex, 4 g daily. Tetracycline mouthwashes are used during an attack.

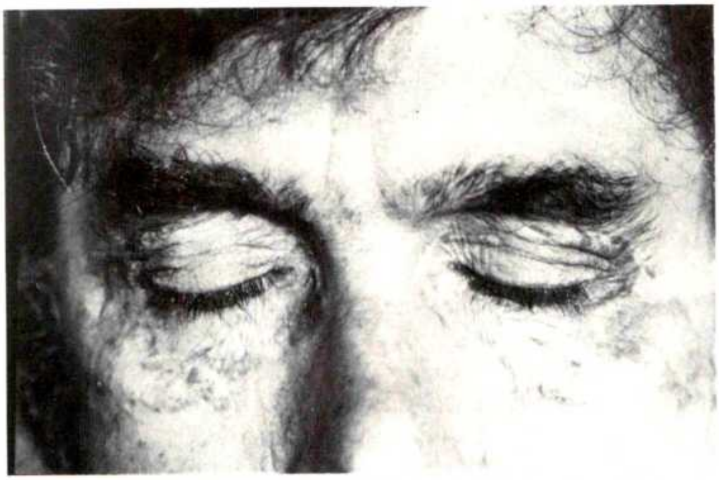

FIGURE 1. Extensive plaques of xanthelasma encircling the eyelids.

## (46) Xanthelasma

DR R.E.CHURCH

D.S., male, aged 55 years.

*History*. This patient developed jaundice at the age of 10 years which lasted only 1–2 weeks, after which he developed yellow patches round the eyelids. He was again jaundiced at the age of 23 years and the yellow patches extended. He has been variously treated with destructive measures such as trichloracetic acid and diathermy, and has also had plastic surgery to the eyelids. In 1975, he developed lichen planus, and in 1983, sheets of plane xanthoma appeared in the scalp. There is no family history of this condition.

*Examination*. There are very extensive plaques of xanthelasma encircling the eyelids (Fig. 1) and sheets of xanthomata in the scalp.

*Investigations*. Liver function tests and serum lipids have been estimated repeatedly and always found normal. The HBs Ag was not detected and the only abnormality ever found has been a slightly raised IgM of 4·1 g/l, IgG and IgA being normal.

*Comment*. This patient has had very extensive xanthelasma for 45 years without developing any significant systemic disturbance.

## (47) Plane xanthomata and monoclonal gammopathy

DR R.E.CHURCH

G.H., male, aged 48 years.

*History*. This patient developed attacks of urticaria about twice weekly during 1980. He was investigated at the Victoria Hospital, Worksop (Dr Corbett) and found to have a monoclonal gammopathy. In March 1982, he was referred to the Royal Hallamshire Hospital because of persistent itching and was found to have yellow coloured sheets on the trunk and limbs. There was a family history of diabetes in his mother, and his father died of carcinoma of the liver.

*Examination.* He has xanthelasma, and there are sheets of plane xanthoma on his limbs and trunk.

*Investigations.* In 1982, a skeletal survey was normal, bone marrow examination showed no increase in plasma cells and no Bence-Jones protein was detected in the urine. However, IgA was elevated to 10 g/l, and by 1983, this had increased to 19 g/l. Lipoprotein electrophoresis demonstrated no abnormality and there was no lipid staining band corresponding to the monoclonal band seen on protein electrophoresis. Warm plasma showed marked turbidity when standing at room temperature. This was not a true cryoprecipitate as once formed it was extremely insoluble. Biopsy of the skin showed sparse xanthoma cells and occasional lymphocytes round dermal blood vessels. Chromosome studies showed no evidence of leukaemia. In September 1985, he was found to have an iron deficiency anaemia with a haemoglobin of 10·8 g/dl which responded to treatment with oral iron.

*Comment.* The association between myelomatosis and plane xanthoma is well recorded (Taylor *et al.*, 1978). This patient seems to correspond to the patient recorded by Wilson *et al.* (1975) in whom a cryoprecipitate of a paraprotein-lipoprotein complex was demonstrated. It is not uncommon for immune complexes to form cryoprecipitates, and this might explain the deposition of lipids in the skin.

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## (48) Waldenstrom's hyperglobulinaemic purpura associated with Sjogren's syndrome

Drs H.McGrath and D.Slater

*Doncaster Royal Infirmary and Rotherham District General Hospital*

A.G., female, aged 22 years.

*History.* For 3 years this patient has had recurrent painful erythematous swelling of her legs associated with purpura. The erythema resolves after 10 days leaving marked haemosiderotic staining. Her pregnancy (expected date of delivery 4.2.86) has increased the severity of the condition. She complains of a gritty sensation in her eyes. Dry food has been difficult to eat. She has been hoarse for 4 months and has a non-productive cough and wheeze.

(The patient's baby was born on 13 January 1986. Because of his mother's illness he was monitored for 2 days in a special care unit. As physical examination was normal and his electrocardiograph was normal the baby was discharged home. Unfortunately, he died suddenly aged 3 weeks. Death was attributed to 'unexpected death in infancy' as post mortem examination showed no specific cause of death.)

*Examination.* The patient has erythematous macules with purpura on the legs, extending onto her thighs. There is marked haemosiderotic staining of her legs. Her mouth is dry and she has very little tear secretion.

*Investigations.* Her peripheral blood count and renal function were normal. The ESR was 70 mm/h. The globulin was raised at 48 g/l. Liver function tests were otherwise normal. IgG was raised at 30 g/l (normal 5·8–18·5 g/l) at 37°C and 4°C. IgM and IgA were normal at 37°C and 4°C. Ro (SSA) antibody was positive. Other nuclear and cytoplasmic antibodies were negative. RA factor was present at a dilution of 1 in 400. Shirmer's test showed minimal tear secretion. There was a marked increase in IgA, IgG and IgM immune complexes.

*Histology.* Histology of skin shows classical leucocytoclastic vasculitis involving the papillary and reticular dermis with an infiltrate of acute inflammatory cells and eosinophils. Leucocytoclastic vasculitis is a characteristic of the acute lesion in Waldenstrom's hyperglobulinaemic purpura. Immunofluorescence of lesional skin was negative.

*Treatment.* No benefit has been derived from treatment with support bandages and prednisolone 10 mg daily. As her symptoms are distressing, plasmapheresis (Hewitt *et al.*, 1984) is being considered.

*Comment.* Kyle *et al.* (1971) divided Waldenstrom's hyperglobulinaemic purpura into two groups, primary and secondary. In his series, over half of the patients with secondary hyperglobulinaemic purpura had Sjogren's disease or keratoconjunctivitis sicca.

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## (49) Neurofibromatosis

DRS S.S.BLEEHEN AND I.M.STRACHAN

C.N., female, aged 34 years.
L.N., male, aged 13 years.

*History.* Mother and son were referred because they were suspected of having neurofibromatosis. Her father had neurofibromatosis. Over the past 2 years she had developed several subcutaneous tumours. Both patients have had pigmented lesions since birth.

*Examination.* They both had many typical 'café au lait' macules scattered on the trunk and limbs. There were freckles in the axillae of both patients, and the mother had several soft subcutaneous neurofibromata on the trunk. Lisch nodules were present on the irises of both patients.

*Histology (C.N.).* A nodule on the hip was biopsied and showed the histology of a benign neurofibroma. A skin biopsy of a 'café au lait' macule on the lower back showed, on electron microscopy, several typical melanin macroglobules.

*Comment.* The disorder of von Recklinghausen disease is inherited as an autosomal dominant and the risk of occurrence is about one in 3300 births. The presence of more than five 'café au lait' macules associated with axillary freckling is sufficient to suspect the diagnosis even before tumours develop (Riccardi, 1981). Another important diagnostic sign is the presence of pigmented tumours on the iris–Lisch nodules. These are present in 94% of patients with neurofibromatosis over the age of 6 years and are frequently seen in younger patients (Lewis & Riccardi, 1981). Melanin macroglobules are spherical cytoplasmic granules, often several microns in diameter, and are found in the melanocytes, particularly in the 'café au lait' macules (Nakagawa *et al.*, 1984).

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## (50) Leiomyoma cutis

DRS W.F.G.TUCKER AND C.I.HARRINGTON

A.B., female, aged 30 years.

*History*. This patient first noticed a rash affecting her leg at the age of 21 years. There are no symptoms, but the appearance is unsightly unless she is suntanned. There has been a gradual extension of the area affected. No other members of her family are affected.

*Examination*. Small brownish-pink dermal papules are arranged in a linear distribution from the dorsum of the left foot up the anterolateral aspect of the left shin (Fig. 1) and calf to the thigh, and the lateral aspect of the buttock. A 'café au lait' macule is present on the left thigh.

*Histology*. Shows the characteristic appearance of a leiomyoma, demonstrating a spindle cell neoplasm with the staining properties of muscle cells, arising from an arrector pili muscle.

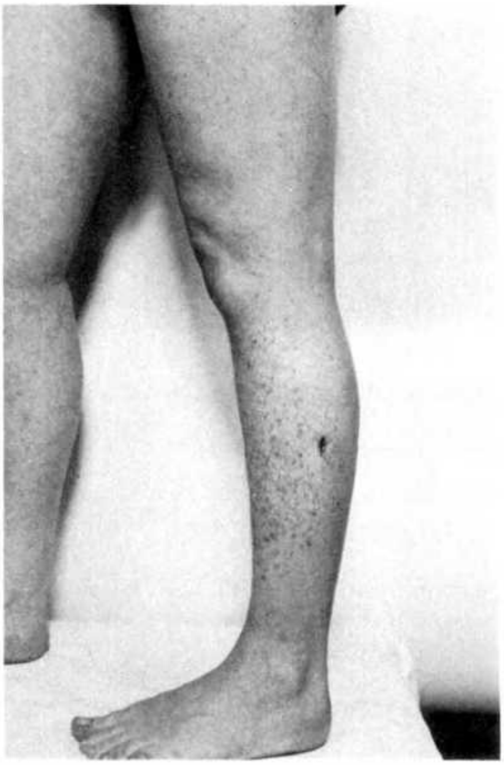

FIGURE 1. Brownish-pink dermal papules of leiomyoma cutis.

*Comment.* The unilateral distribution of the multiple leiomyomata in this patient, and the localization to a single limb, is strongly suggestive of a naevoid origin.

## (51) Angio-leiomyoma

DRS M.L.WOOD AND R.E.CHURCH

J.H., female, aged 50 years.

*History.* This patient was noted to have a slightly tender lumpy area over the lateral border of the right foot, when attending the orthopaedic clinic with low back pain in 1982. She had first noticed it about 3 years prior to that date, and it has slowly increased in size since then, to such an extent that it now interferes with her footwear.

*Examination.* There are flesh coloured nodules on the right lateral sole with a cobblestone appearance and rubbery consistency (Fig. 1).

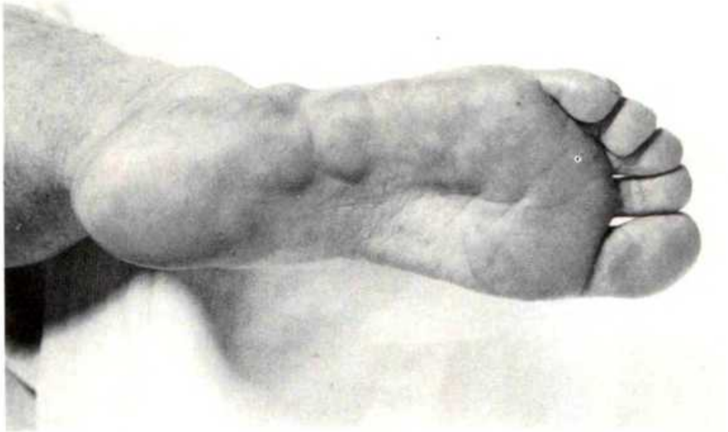

FIGURE 1. Irregular nodular swelling over the lateral border of the right foot.

*Histology.* A section shows benign angiomatous lesions, with areas of myxoid change and smooth muscle, consistent with an angio-leiomyoma. There is nothing to suggest Kaposi's sarcoma or glomus tumour.

*Treatment.* In view of the benign nature of this lesion, it was decided in conjunction with the plastic surgeons, that she should be managed conservatively and has therefore been supplied with surgical shoes and a weight-reducing diet.

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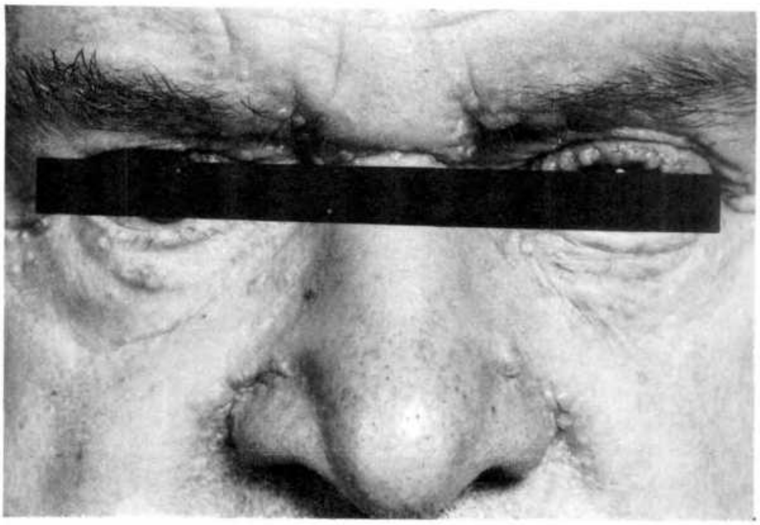

FIGURE 1. Multiple tricho-epitheliomata around the eyes.

## (52) Tricho-epitheliomata with basal cell carcinomata

DR R.E.CHURCH

A.W., male, aged 62 years.

*History*. In about 1973, this patient developed a slowly enlarging nodule on the left eyelid followed by more lesions on the eyelids and central facial panel. The nodule on the eyelid was found histologically to be a basal cell carcinoma; that and other enlarging nodules have been treated with X-rays or excision. There is no family history of this condition.

*Examination*. There are numerous dome-shaped nodules on the eyelids (Fig. 1), bridge of the nose and the naso-labial folds.

*Histology*. A biopsy of the small nodules on the central facial panel confirms the diagnosis of tricho-epithelioma.

*Comment*. Histologically, there is no sharp boundary between tricho-epithelioma and basal cell carcinoma, but lesions which steadily enlarge or ulcerate should be treated as basal cell carcinomata.

## (53) Gorlin's syndrome

DRS W.F.G.TUCKER AND C.I.HARRINGTON

U.B., female, aged 41 years.

*History*. The patient came to the clinic in July 1979 at the age of 34 years, complaining that a long-standing 'mole' on her face had begun to bleed after washing. She recalled on questioning that dental cysts had been removed in her 20s. The patient's son, P.L., aged 13 years, has recently had two large dental cysts excised from his mandible.

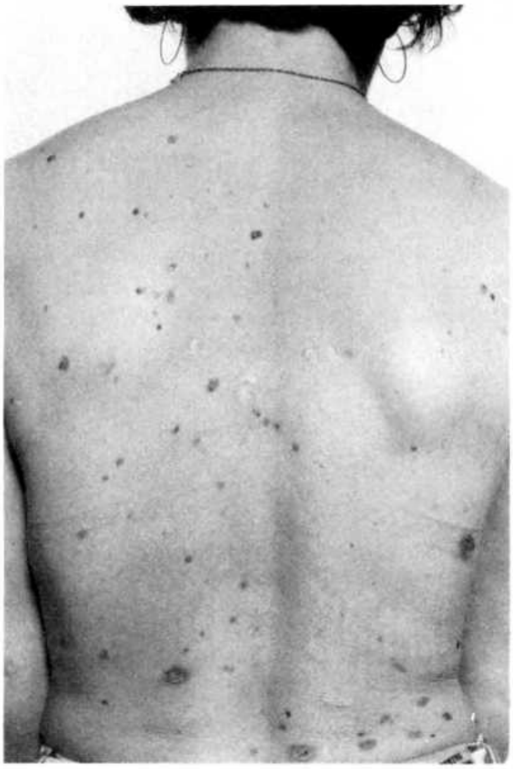

FIGURE 1. Multiple basal cell epitheliomata over the back.

*Examination.* A large number of basal cell epitheliomata are present over her face, neck and trunk (Fig. 1). Palmar pits are present and there is some degree of frontal bossing, together with a wide nasal base. The patient's son has similar facial features, but only a few pits on the left palm.

*Investigations.* The chest X-ray and skull X-ray were normal. The orthopantomograph showed a root remnant in the right lower region. The orthopantomograph on P.L. showed two large cysts in both angles of the mandible.

*Histology.* A biopsy from a typical lesion excised in 1983 shows a nodule of basaloid cells at the dermo-epidermal junction, having some differentiation towards squamous cells and showing basal invasion of the superficial dermis, typical of a basal cell carcinoma.

*Treatment.* Topical 5% fluorouracil cream has proved effective for many of the smaller superficial lesions, while curettage and cautery, cryosurgery, simple excision and skin grafting have all been employed for larger lesions, depending on the site.

*Comment.* This patient is a typical example of the basal cell naevus syndrome. Although her parents were unaffected, her son also has early signs of involvement, demonstrating the dominant inheritance of this condition.

## (54) Bazex syndrome

DR R.E.CHURCH

B.S., female, aged 38 years.
J.S., male, aged 8 years (son of B.S.)

*History*. B.S. developed lesions on the face at the age of 21 years. In 1971, a biopsy confirmed the diagnosis of multiple basal cell carcinomata and these have been variously treated by curettage, excision and radiotherapy. Her father and sister have similar lesions.

Her son, J.S., born in 1977, developed profuse milia on the face in infancy followed by milia and subsequent follicular atrophy on the backs of the hands. He has an older sister who has no abnormality.

*Examination*. B.S. has scattered nodules and radiotherapy scars on the face, with sparse lesions of milia. There are atrophic pits on the backs of the hands. She has a congenital cataract in the right eye. J.S. has fair hair with hypotrichosis, milia on the face, and atrophic pits and milia on the backs of the hands. He has also developed calcinosis of the pinnae.

*Investigations*. X-rays of B.S. show spina bifida occulta.

*Comment*. Milia are said to be a feature of this syndrome in some reports, and in this case are the earliest manifestations.

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## (55) Birt-Hogg-Dubé syndrome and lichen sclerosus et atrophicus

DRS C.I.HARRINGTON AND A.G.MESSENGER

M.A., female, aged 64 years.

*History*. This patient first attended the clinic in 1980 with a 40-year history of small swellings on her face, neck, chest and arms. She requested removal of the enlarging lesions on her face. She had a thyroidectomy for thyrotoxicosis in 1964.

*Family history*. Her mother and two maternal aunts were similarly affected. One aunt had vitiligo.

*Examination*. She has numerous follicular papules on her face, neck and anterior chest, and axillae (Fig. 1). There are also skin tags in the axillae, and on the neck and ante-cubital fossae.

In 1983, she developed lichen sclerosus et atrophicus (LSA) of the vulva.

*Investigations*. The full blood count, ESR, blood glucose, thyroid function tests, and serum vitamin B12 were all normal. Tissue antibodies were negative.

*Histology*. The lesions removed have been fibrofolliculomas and achrochordons. Vulval biopsy confirmed lichen sclerosus et atrophicus.

*Treatment*. Troublesome lesions have been removed surgically. Her lichen sclerosus et atrophicus has responded well to topical steroid therapy.

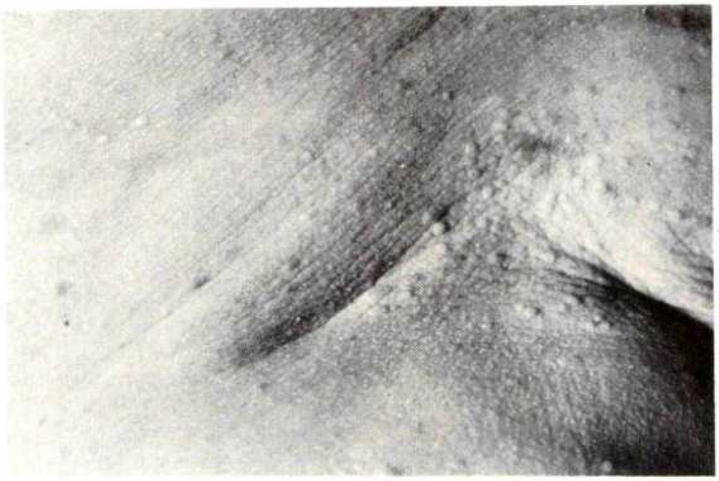

FIGURE 1. Fibro-folliculomas around the axilla.

*Comment.* This patient's lesions fit the description described by Birt, Hogg and Dubé (1977). The occurrence of lichen sclerosus et atrophicus in a patient with this syndrome has not been recorded previously. This patient has the recognized personal and family history of auto-immune disease in association with LSA (Harrington & Dunsmore, 1981).

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## (56) Hidradenomes eruptif

DRS S.B.BITTINER AND C.I.HARRINGTON

I.C., male, aged 28 years.

*History.* The patient developed a rash consisting of discrete brown lesions on his lower abdomen 10 years ago. It has since spread towards his axillae and down onto his thighs. The rash is asymptomatic. There is no relevant family history.

*Examination.* There are discrete brown, non-scaley papules extending from the thighs to the upper trunk, mainly on the anterior aspect of the body (Fig. 1).

*Histology.* Sections show a benign syringoma with surrounding chronic inflammation. Mast cells are present in normal numbers.

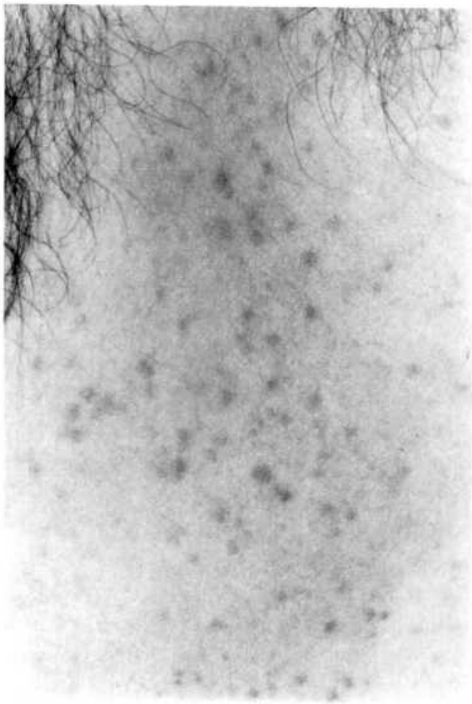

FIGURE 1. Benign syringomata on the anterior chest.

## (57) Steatocystoma multiplex suppurativa and pachyonychia congenita in two siblings

DR M.E.KESSELER

Rotherham District General Hospital

D.A., male, aged 47 years.
M.M., female, aged 51 years.

*History*. D.A. has had severe steatocystoma multiplex for 40 years. In 1955, he had left axillary resection and graft. Since 1980, he has had frequent painful, suppurative episodes, many lesions being incised in Casualty. These have been worst in areas of friction on his lower trunk.

M.M., the sister of D.A., has less severe and non-inflammatory steatocystoma. In addition, since 1975, she has developed thickening of the fingernails.

The father of D.A. and M.M. had similar cysts.

*Examination*. D.A. shows severe, extensive cyst formation, several cysts being inflamed, but the left axillary graft area is cyst-free. Numerous dilated, plugged and scarred follicles are present, and both hallux nail plates are thickened.

M.M. shows less extensive cyst formation. Her nail dystrophy, however, is more marked, with gross finger nail plate thickening, and hyperkeratosis of the ball of the right foot is present.

*Investigations*. Histology in D.A. was that of steatocystoma multiplex. Hallux mycology in D.A. showed a dermatophyte fungus.

*Treatment*. D.A. did not respond to a 4-month course of Ro-accutane® 1 mg/kg (Dr Cunliffe, Leeds, 1981). Long-term antibiotic therapy was re-started recently after several years' interval, with Augmentin®. The response has been good (February, 1986) and treatment with etretinate deferred.

*Comments*. M.M.'s nail dystrophy and plantar hyperkeratosis suggest that this family may have pachyonychia congenita (Samman Type III) associated steatocystoma multiplex.

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SODERQUIST *et al*. (1968) Pachyonychia congenita with epidermal cysts and other congenital dyskeratoses. *Archives of Dermatology*, **97**, 31.

## (58) Candida-endocrinopathy syndrome. Squamous cell carcinoma of the tongue

DRS A.G.MESSENGER AND C.I.HARRINGTON

J.N., female, aged 50 years.

*History*. At the age of 9 years, this patient developed oral candidiasis. In 1955, Addison's disease was diagnosed, and shortly afterwards she developed a candidal nail dystrophy (Fig. 1). The oral and nail candidiasis persisted in spite of adequate adrenal replacement therapy and local anti-fungal treatment. In 1975, she had an episode of alopecia areata. Since 1981, she has taken ketoconazole 200 mg daily. Her nails rapidly improved and became normal within 6 months of starting treatment. However, white, adherent plaques persisted on the tongue. Early in 1985, she was treated with transfer factor in an attempt to clear the tongue. This was unsuccessful, although the previously negative reaction to intradermal candida antigen converted to positive. In June 1985, an indurated nodule appeared on the dorsum of the tongue. This was excised by the plastic surgeons.

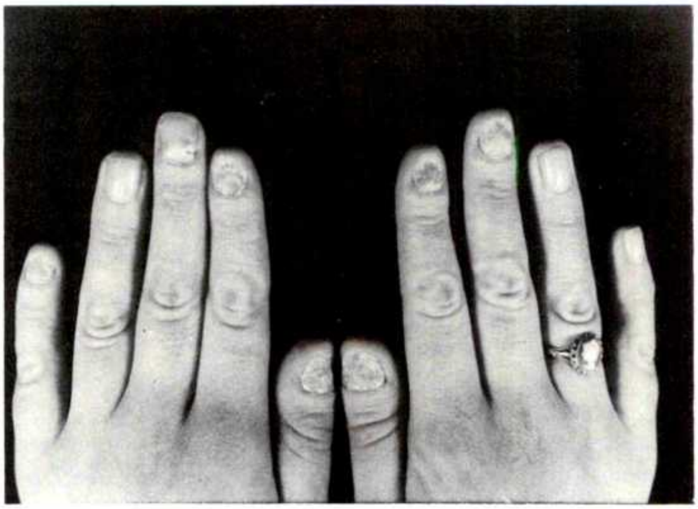

FIGURE 1. Candida nail dystrophy in 1955.

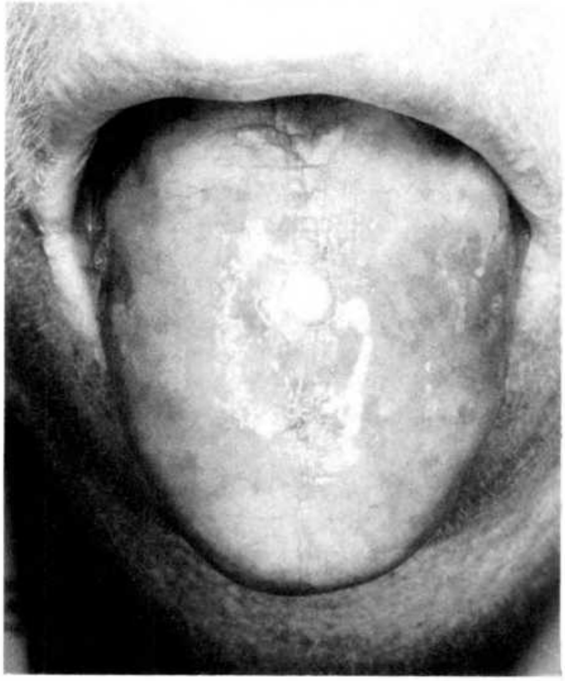

FIGURE 2. Candidiasis of the tongue showing thickened white plaques and atrophic areas, and a central indurated nodule.

*Examination*. There are irregular areas of white, thickened mucosa on the dorsum of the tongue with intervening reddish atrophic areas (Fig. 2). The nails are normal.

*Investigations*. Full blood count and serum iron were normal. The peripheral blood total lymphocyte count is normal but B cell numbers are depressed ($0{\cdot}04 \times 10^9/l$). T cell numbers are normal and show a normal response to PHA, but an absent response to candida antigen.

*Histology*. Tongue biopsies in 1982 and 1983 revealed a thickened, non-dysplastic epidermis with candida hyphae on the surface. These features are consistent with chronic hyperplastic candidiasis. The lesion excised from the tongue in 1985 was a well differentiated, micro-invasive squamous cell carcinoma.

*Comment*. This patient has chronic mucocutaneous candidiasis associated with an endocrinopathy. As in this case, hormone replacement therapy does not usually influence the candidiasis. Ketoconazole has been a major advance in the treatment of chronic mucocutaneous candidiasis, although the response is not always complete. The development of oral squamous cell carcinoma is a recognized, albeit uncommon, complication of chronic hyperplastic candidiasis (Cawson, 1968; Eyre & Nally, 1971).

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EYRE, J. & NALLY, F.F. (1971) Oral candidosis and carcinoma. *British Journal of Dermatology*, **83**, 73.

## (59) Non-iatrogenic chronic arsenicism

DRS M.E.KESSELER AND D.SLATER

*Rotherham District General Hospital*

R.C., male, aged 37 years.

*History.* This patient presented in April 1984 with a 10-year history of pigmented lesions of the trunk and palmoplantar 'warts'. In childhood he lived in Antofagasta, Chile, emigrating to England in 1977. Between 1958 and 1970, this town received a water supply with a high arsenic content (0·8 ppm).

*Examination.* At presentation, he had numerous pigmented basal cell carcinomata, predominantly on the trunk, palmoplantar arsenical keratoses and raindrop pigmentation.

*Investigations.* Pigmented lesion histology confirmed basal cell carcinomata. Arsenic was not identified on lesional skin and hair X-ray microanalysis, but hair mineral analysis confirmed 0·62 ppm arsenic in the hair tips. It was remarked that this was the highest level recorded in the preceding 3200 hair arsenic analysis samples, and was three times the average 'normal' level.

*Treatment.* Fourteen basal cell carcinomata have been excised under local and general anaesthetic to date. In September 1985, etretinate was prescribed and the patient is currently maintained on 25 mg daily. There has been symptomatic improvement in his keratoses and a probable reduction in the rate of growth of his rodent ulcers.

*Comment.* Most hair analysis studies in water contamination arsenicism have shown higher levels than in this patient. This may, however, reflect a delay in sampling. Since installation in 1970 of a plant to purify the River Tocance, population mean hair arsenic levels have fallen.

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## (60) Anetoderma of Jadassohn

DR A.WALKER

*Chesterfield and North Derbyshire Royal Hospital*

J.S., female, aged 29 years.

*History.* Over the last 3 years, this patient has developed 'blemishes' on her arms, the 'V' of the neck and back of the neck. She has no symptoms, the only problem being the cosmetic appearance. Her parents, three sisters, three brothers and two children have no skin problems.

*Examination.* There is anetoderma affecting the arms, neck and lower face. The overlying skin is normal in colour, but a little atrophic and wrinkled, and the underlying tissue pouches outwards.

*Histology.* The epidermis is thin and hypertrophic. Elastic stain shows marked diminution of the dermal elastic tissue.

## (61) Dowling-Degos disease

DR M.E.KESSELER

*Rotherham District General Hospital*

G.C., male, aged 55 years.

*History.* This patient presented with a 5-year history of axillary and perineal pigmentation. He is unaware of any relevant family history.

*Examination.* A macular reticulate pigmentation was present in both axillae, groin and peri-anal areas. Follicular plugging and scarring was seen below the lower lip.

*Investigations.* Histology showed basiloid downgrowths into the papillary dermis, containing increased pigmented keratinocytes and melanocytes and small keratin cysts, consistent with reticulate pigmented anomaly of Dowling-Degos disease.

### REFERENCE

WILSON-JONES, E. & GRICE, K. (1977) Reticulate pigmented anomaly of the flexures. *Archives of Dermatology*, **114**, 1150.

## (62) Atypical dermal melanocytic naevus

DRS M.E.KESSELER AND D.SLATER

*Rotherham District General Hospital*

L.B., female, aged 19 years.

*History.* The patient presented with a 3-year history of left ear and neck pigmentation.

*Examination.* Areas of diffuse green/blue pigmentation were seen on the left lower pinna, retro-auricular and supra-clavicular areas. There was focal darker pigmentation within these areas.

*Investigations.* Patch tests to the standard battery, gold salts and cosmetics showed a positive allergic reaction to nickel only.

*Histology.* Retro-auricular skin biopsy showed a mild dermal lymphohistiocytic infiltrate, with occasional exocytosis and focal spongiosis. Spindle-shaped pigment cells were present in and below the deep dermis. Electron microscopy showed these to be a mixture of melanophages and melanocytes. X-ray micrograph analysis identified magnesium silicates in the upper epidermis and iron, nickel and chromium in the lower epidermis. Sulphur and aluminium granules were present in dermal macrophages.

*Comment.* The pigmentation has been shown to be due to a dermal melanocytic naevus. Comparative X-ray micrograph studies have shown sulphur in common blue naevi. Retro-auricular naevus of Ota is well documented (Hidano *et al.*, 1967), but the superficial inflammatory component in this patient is unusual. It is improbable that sub-clinical nickel dermatitis has contributed to the pigmentation.

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## (63) Peutz-Jeghers syndrome without polyposis

DR C.I.HARRINGTON

M.L., female, aged 82 years.

*History.* This patient came to the clinic in August 1984 concerned about freckles on her knuckles which had appeared over the previous few weeks. She stated that freckles had been developing on her lips over the last 10–20 years. She was otherwise well apart from chronic simple glaucoma, and had never had any bowel problems. She had no relatives with abnormal pigmentation or bowel symptoms.

*Examination.* She has lentigines on the lips, oral mucosa and knuckles.

*Comment.* Pigmentary changes indistinguishable from those in Peutz-Jeghers syndrome may occur sporadically in adults without intestinal polyposis (Calnan, 1960).

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CALNAN, C.D. (1960) The Peutz-Jeghers syndrome. *Transactions A. Rep. St John's Hospital Dermatological Society, London*, **44**, 58.

*(Contents continued from back cover)*

Oral nifedipine treatment for Raynaud's phenomenon secondary to systemic sclerosis—a double-blind, placebo-controlled study; R.H.MEYRICK THOMAS, M.RADEMAKER, S.M.GRIMES, I.B.KOVAKS, A.MACKAY, S.BOWCOCK, E.D.COOKE AND J.D.T.KIRBY 37
Cutaneous endometriosis: a histopathological study; M.J.TIDMAN AND D.M.MACDONALD 38
Epidermal Class II HLA expression in atopic dermatitis; J.N.W.N.BARKER AND D.M.MACDONALD 38
The detection of extracellular antigens in formaldehyde-fixed paraffin-embedded cutaneous tissue; R.CERIO, M.H.ALLEN AND D.M.MACDONALD 39
Treatment of common and plantar viral warts with human lymphoblastoid interferon-α; J.R.GIBSON, S.G.HARVEY, D.KEMMETT AND J.SALISBURY 40
Clinical and laboratory manifestations of cutaneous virilism; J.H.BARTH, C.A.CHERRY, R.P.R.DAWBER AND F.WOJNAROWSKA 40
Contact sensitivity in community-based leg ulcer patients; M.KULOZIK, S.POWELL, T.J.RYAN AND G.CHERRY 41
Ehlers-Danlos syndrome type VIII; D.A.FENTON, F.M.POPE, A.TEMPLE, W.A.GALLOWAY, G.GASSON, M.M.BLACK AND J.D.WILKINSON 42

**Case reports**

Focal facial dermal dysplasia (Brauer's syndrome); C.I.HARRINGTON 43
Focal dermal hypoplasia (Goltz's syndrome); S.B.BITTINER AND R.E.CHURCH 44
Focal dermal hypoplasia (Goltz's syndrome) with extensive capillary haemangioma; S.S.BLEEHEN 44
Tricho-rhino-phalangeal syndrome; S.E.THOMAS, J.MAHOOD AND R.E.CHURCH 45
Incontinentia pigmenti. Bloch-Sulzberger syndrome; S.S.BLEEHEN AND M.L.WOOD 47
Incontinentia pigmenti achromians (hypomelanosis of Ito); S.S.BLEEHEN, S.B.BITTINER AND B.L.PRIESTLEY 48
Klippel-Trenaunay-Parkes-Weber syndrome; W.F.G.TUCKER AND C.I.HARRINGTON 49
Cutis marmorata telangiectatica congenita; W.F.G.TUCKER AND S.S.BLEEHEN 50
Cutis marmorata telangiectatica congenita; S.B.BITTINER AND R.E.CHURCH 51
Alagille's syndrome (arteriohepatic dysplasia); D.M.LISTER, D.SHUTTLEWORTH, R.A.C.GRAHAM-BROWN AND S.TANNER 52
Livedo reticularis and cerebrovascular lesions; S.E.THOMAS, R.E.CHURCH AND G.A.B.DAVIES-JONES 53
Discoid lupus erythematosus; S.E.THOMAS AND R.E.CHURCH 54
Lupus erythematosus profundus; A.E.WALKER 55
Sub-acute cutaneous lupus erythematosus and malabsorption; A.G.MESSENGER AND R.E.CHURCH 56
Protein losing enteropathy due to SLE; M.L.WOOD AND R.E.CHURCH 58
Lichen amyloidosis; S.E.THOMAS AND R.E.CHURCH 59
Primary amyloidosis; S.B.BITTINER AND C.I.HARRINGTON 61
Systemic mastocytosis; W.F.G.TUCKER AND C.I.HARRINGTON 62
Systemic mastocytosis; W.F.G.TUCKER AND C.I.HARRINGTON 64
Reticular erythematous mucinosis (R.E.M. syndrome); R.E.CHURCH 65
Darier's disease; A.G.MESSENGER FOR R.E.CHURCH 67
Erythrokeratoderma variabilis (Mendes da Costa's syndrome); W.F.G.TUCKER AND C.I.HARRINGTON 67
Erythrokeratoderma variabilis (Mendes da Costa's syndrome); W.F.G.TUCKER AND C.I.HARRINGTON 68
Lymphomatoid papulosis and poikiloderma; S.B.BITTINER AND R.E.CHURCH 68
Pityriasis lichenoides developing into mycosis fungoides; S.S.BLEEHEN AND D.N.SLATER 69
Pre-reticulotic eruption with alopecia; D.M.LISTER AND R.A.C.GRAHAM-BROWN 71
Lymphomatoid granulomatosis; M.L.WOOD AND C.I.HARRINGTON 72
Cutaneous sarcoidosis; S.E.THOMAS AND R.E.CHURCH 75
Sarcoidosis; S.E.THOMAS AND C.I.HARRINGTON 76
Sarcoidosis; A.G.MESSENGER AND S.S.BLEEHEN 77
Granulomatous cheilitis; S.E.THOMAS AND S.S.BLEEHEN 78
Cutaneous Crohn's disease; S.E.THOMAS AND R.E.CHURCH 79
Crohn's disease; S.E.THOMAS, R.E.CHURCH AND C.D.HOLDSWORTH 80
Atrophic morphoea; W.F.G.TUCKER AND R.E.CHURCH 81
Diabetes, necrobiosis lipoidica and granuloma annulare; M.L.WOOD, S.S.BLEEHEN AND J.D.WARD 81
Granuloma annulare; W.F.G.TUCKER AND S.S.BLEEHEN 82
Benign familial pemphigus (Hailey-Hailey disease); W.F.G.TUCKER AND S.S.BLEEHEN 83
Parasitophobia and linear IgA disease; S.E.THOMAS AND R.E.CHURCH 83
Epidermolysis bullosa simplex. Mental and physical handicap; C.I.HARRINGTON 84
Two siblings with epidermolysis bullosa; M.L.WOOD, R.E.CHURCH AND M.E.KESSELER 85
Recessive dystrophic epidermolysis bullosa; M.L.WOOD AND C.I.HARRINGTON 86
Dominant dystrophic epidermolysis bullosa (Pasini variant); M.L.WOOD AND C.I.HARRINGTON 86
Gunther's disease (congenital erythropoietic porphyria); C.I.HARRINGTON 87
Bullous eruption on haemodialysis (pseudoporphyria); M.L.WOOD AND C.I.HARRINGTON 88
Persistent erythema multiforme; S.B.BITTINER AND R.E.CHURCH 89
Xanthelasma; R.E.CHURCH 90
Plane xanthomata and monoclonal gammopathy; R.E.CHURCH 90
Waldenstrom's hyperglobulinaemic purpura associated with Sjogren's syndrome; H.MCGRATH AND D.SLATER 91
Neurofibromatosis; S.S.BLEEHEN AND I.M.STRACHAN 92
Leiomyoma cutis; W.F.G.TUCKER AND C.I.HARRINGTON 93
Angio-leiomyoma; M.L.WOOD AND R.E.CHURCH 94
Tricho-epitheliomata with basal cell carcinomata; R.E.CHURCH 95
Gorlin's syndrome; W.F.G.TUCKER AND C.I.HARRINGTON 95
Bazex syndrome; R.E.CHURCH 97
Birt-Hogg-Dubé syndrome and lichen sclerosus et atrophicus; C.I.HARRINGTON AND A.G.MESSENGER 97
Hidradenomes eruptif; S.B.BITTINER AND C.I.HARRINGTON 98
Steatocystoma multiplex suppurativa and pachyonychia congenita in two siblings; M.E.KESSELER 99
Candida-endocrinopathy syndrome. Squamous cell carcinoma of the tongue; A.G.MESSENGER AND C.I. HARRINGTON 100
Non-iatrogenic chronic arsenicism; M.E.KESSELER AND D.SLATER 102
Anetoderma of Jadassohn; A.WALKER 102
Dowling-Degos disease; M.E.KESSELER 103
Atypical dermal melanocytic naevus; M.E.KESSELER AND D.SLATER 103
Peutz-Jeghers syndrome without polyposis; C.I.HARRINGTON 104

# British Journal of Dermatology, Volume 115, Supplement 30, July 1986

CONTENTS PAGE

Foreword 1

Programme of Meeting 5

**Summaries of papers**

Experience with etretinate for 'PUVA-dependent' psoriatics; R.A.LOGAN AND W.A.D.GRIFFITHS 11
The role of alcohol consumption in the natural history of psoriasis; B.E.MONK AND S.M.NEILL 11
Ultrasound and radio-isotope scans are poor indicators of liver damage in psoriatic patients treated with methotrexate; DEBORAH M.MITCHELL, R.J.JOHNSON, H.J.TESTA, N.Y.HABOUBI AND R.J.G.CHALMERS 13
Pulmonary function in patients on long-term, low-dose methotrexate; T.J.PHILLIPS, P.WALLIS AND H.BAKER 13
Safety of photochemotherapy—an eight-year follow-up study; N.H.COX, D.J.DOWNEY, E.J.TUYP, S.K.JONES, J.L.JAY, H.MOSELEY AND RONA M.MACKIE 14
The epidemiology of *Trichosporon beigelii* infection in Houston, Texas; DEBRA CHESTER KALTER 15
The role of anaerobic bacteria in the pathogenesis of tropical (phagedenic) ulcer; B.ADRIAANS, B.DRASAR AND R.HAY 15
Dermatological conditions associated with HTLV-III virus infection; M.M.WALKER, C.E.M.GRIFFITHS, J.N.LEONARD, A.V.POWLES, J.WEBER, W.HARRIS AND R.J.CLAYTON 16
Pityriasis lichenoides-like lesions associated with poikiloderma: a precursor of mycosis fungoides; J.K.RIVERS, P.D.SAMMAN, M.F.SPITTLE AND N.P.SMITH 17
Photochemotherapy for cutaneous T-cell lymphoma—the St John's experience; R.A.LOGAN, MARGARET F.SPITTLE AND N.P.SMITH 17
Lymphomatoid papulosis: a rare manifestation of the hypereosinophilic syndrome; S.WHITTAKER, C.J.F.SPRY AND R.RUSSELL JONES 18
An investigation into the incidence of auto-immune disorders in patients with localized morphoea; C.I.HARRINGTON AND I.R.DUNSMORE 19
Ocular and oral involvement in bullous and cicatricial pemphigoid: a clinical and immunopathological study; V.A.VENNING, P.A.FRITH, A.J.BRON, P.R.MILLARD AND F.WOJNAROWSKA 19
Dystrophic epidermolysis bullosa: monoclonal antibodies reveal heterogeneity within clinical groups; A.H.M.HEAGERTY, M.J.TIDMAN, I.M.LEIGH, A.JASSIM AND R.A.J.EADY 20
Diabetic sclerodactyly; PAULINE M.DOWD, I.C.GAYWOOD, A.B.KURTZ AND M.E.SHIPLEY 21
Allografts of cultured keratinocytes in clinical practice; T.PHILLIPS, I.M.LEIGH, M.HACKETT, A.BRAIN, P.PURKIS AND H.NAVSARIA 21
A clinical and laboratory study of delayed pressure urticaria; J.S.DOVER, A.KOBZA BLACK AND M.W.GREAVES 22
Recurrence rates of basal cell carcinomas according to site, method of removal, histological type and adequacy of excision; A.W.MACFARLANE, R.K.CURLEY AND R.M.GRAHAM 23
Modes of spread of basal cell carcinoma; N.A.SWANSON, C.B.ZACHARY AND R.C.GREKIN 23
Adapting Mohs' micrographic surgery to the needs and resources of the National Health Service; M.G.C.DAHL AND A.J.MALCOLM 24
Incontinentia pigmenti achromians of Ito. A neurocutaneous disorder; S.S.BLEEHEN, S.B.BITTINER AND B.L.PRIESTLEY 24
Lyell's syndrome—Créteil's experience in 87 cases; J.C.ROUJEAU, C.M.E.ROWLAND PAYNE, J.C.GUILLAUME, D.PENSO, P.SAIAG, J.REVUZ AND R.TOURAINE 25
Prognostic factors in allergic contact dermatitis; S.E.THOMAS AND S.B.BITTINER 25
Allergic contact dermatitis from 1-methylquinoxalinium-*p*-toluenesulphonate in a lithographic printing conditioner; J.S.C.ENGLISH, I.R.WHITE AND R.J.G.RYCROFT 26
The importance of measuring the ankle/brachial systolic pressure ratio in the management of leg ulcers; M.KULOZIK, G.W.CHERRY AND T.J.RYAN 26
A double-blind study of the effect of topical mupirocin in chronic atopic dermatitis; ROSEMARY LEVER, D.DOWNEY, KAY HADLEY, LYN BABER, C.HANNIGAN AND RONA MACKIE 27

**Summaries of posters**

Localization of bullous pemphigoid antibody—an indirect immunofluorescence study of 212 cases using a split-skin technique; R.A.LOGAN, B.BHOGAL, A.K.DAS, P.H.MCKEE AND M.M.BLACK 29
The aminoterminal peptide of type III procollagen as a serological marker of fibrosis in psoriatic patients treated with methotrexate; DEBORAH M.MITCHELL, A.SMITH, BRENDA P.ROWAN, T.W.WARNES, N.Y.HABOUBI AND R.J.G.CHALMERS 29
Increased calmodulin levels in psoriasis: cause or effect? W.F.G.TUCKER, S.MACNEIL, R.A.DAWSON, S.TOMLINSON AND S.S.BLEEHEN 30
Adverse risk factors in atopic eczema: clues from natal interpretation: W.F.G.TUCKER, M.L.WOOD, S.B.BITTINER, S.S.BLEEHEN AND R.E.CHURCH 31
Marie-Unna syndrome; A.G.MESSENGER AND ANNE E.WALKER 32
The cutaneous nodular chain: an unusual pattern of infection; C.MALONEY, M.L.WOOD, R.C.SPENCER AND C.I.HARRINGTON 32
Allergic contact dermatitis from an epoxy compound in a cutting oil; J.S.C.ENGLISH, I.FOULDS, I.R.WHITE AND R.J.G.RYCROFT 33
Effects of topical and oral salbutamol therapy in atopic dermatitis; C.B.ARCHER AND D.M.MACDONALD 33
The use of itraconazole in chronic *Trichophyton rubrum* infections; R.J.HAY, Y.M.CLAYTON AND G.MIDGLEY 34
Further clinical and investigative studies of chlorpromazine phototoxicity; J.FERGUSON, E.M.WALKER AND B.E.JOHNSON 35
Phototoxic plants in Britain; C.R.LOVELL AND GINA MACHIN 35
Micro-computer-based system for contact dermatitis; STEPHANIE SHAW, J.WILKINSON, M.BECK, CAROL GOBEL AND L.CHOW 36
Attitudes to additives—a preliminary report; E.YOUNG, M.PEPPER, J.EVERARD, S.SHAW AND J.D.WILKINSON 36
Solitary morphoea profunda; S.WHITTAKER, N.P.SMITH AND R.RUSSELL JONES 37

*(Contents continued on inside back cover)*

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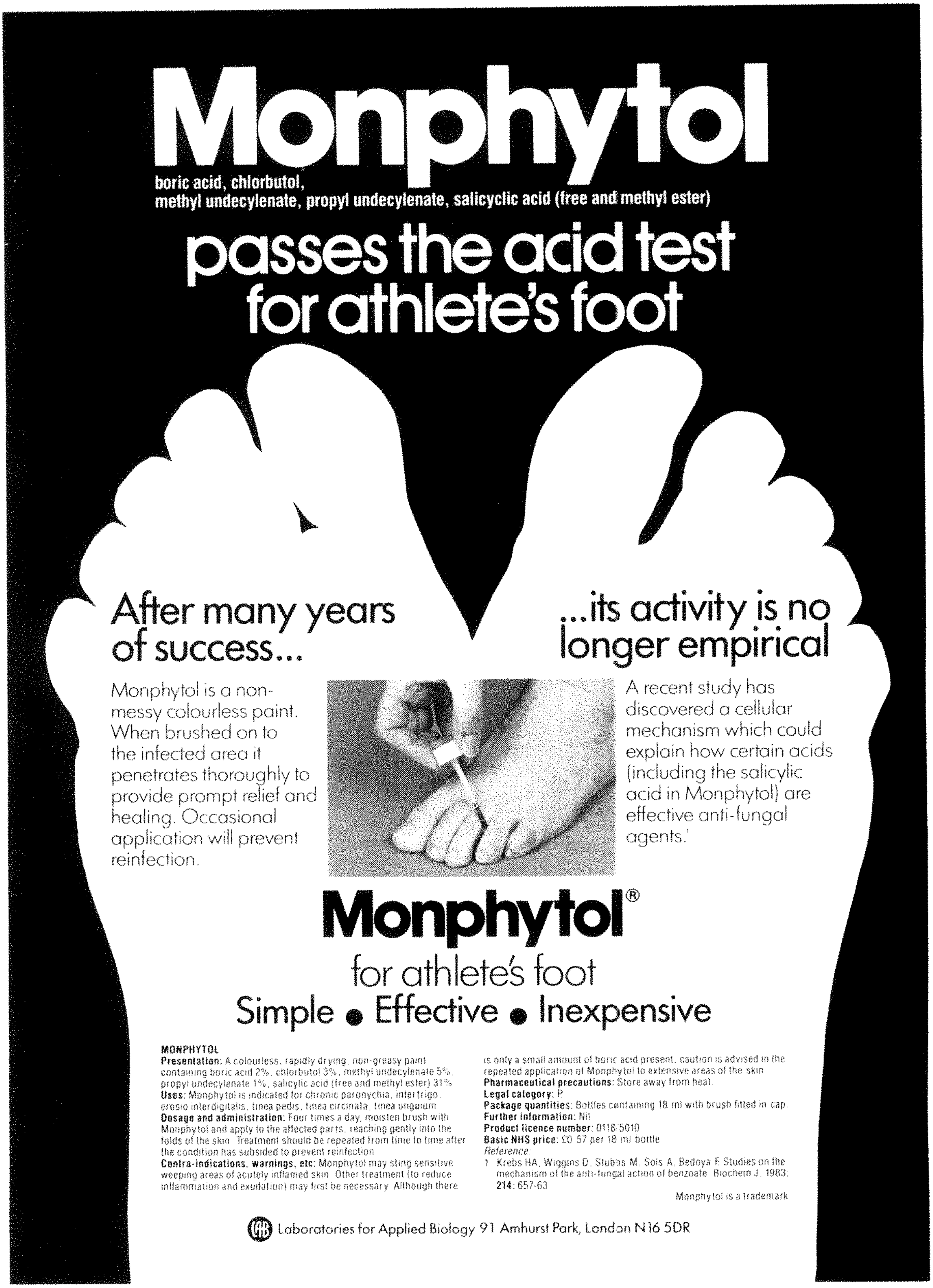

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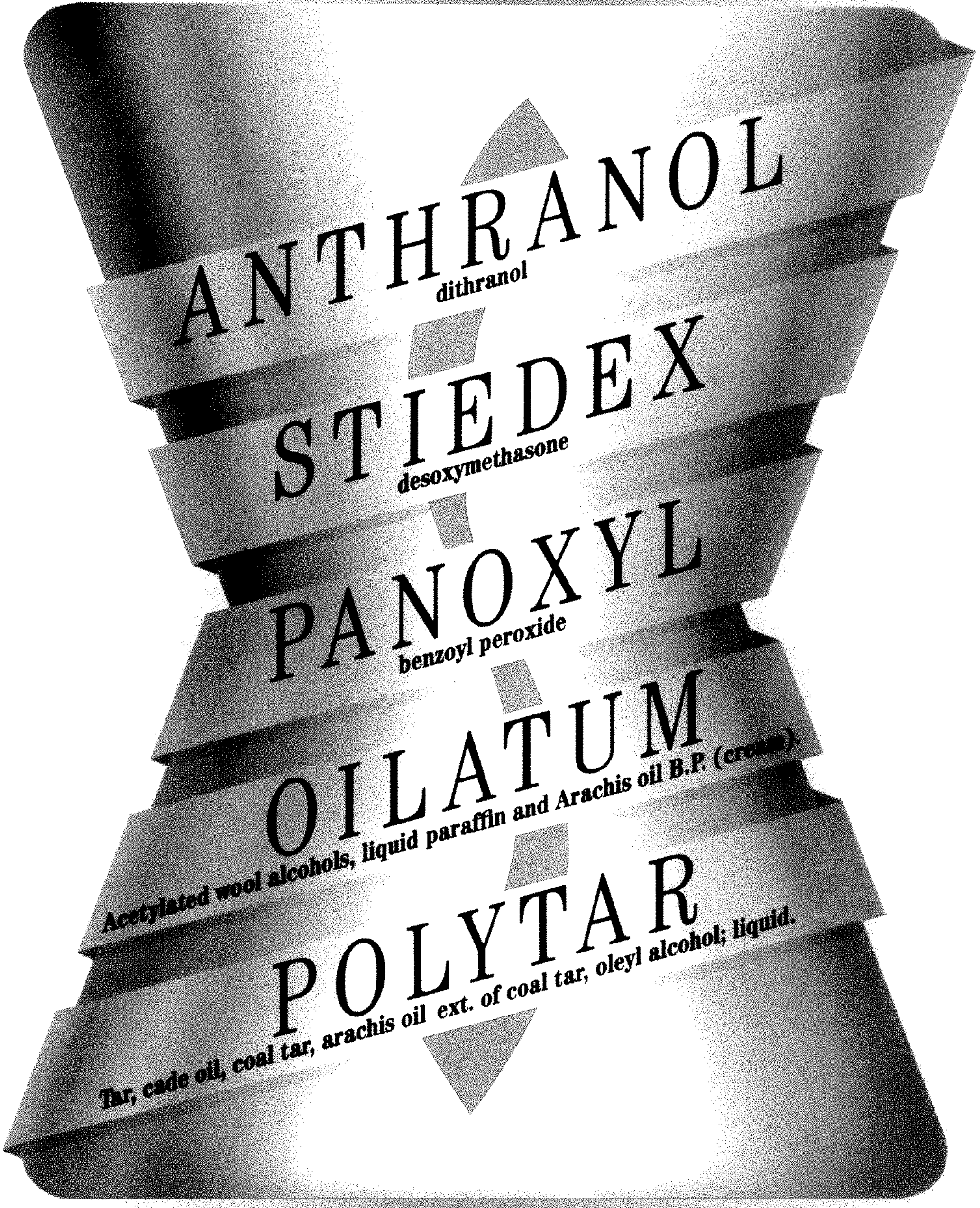
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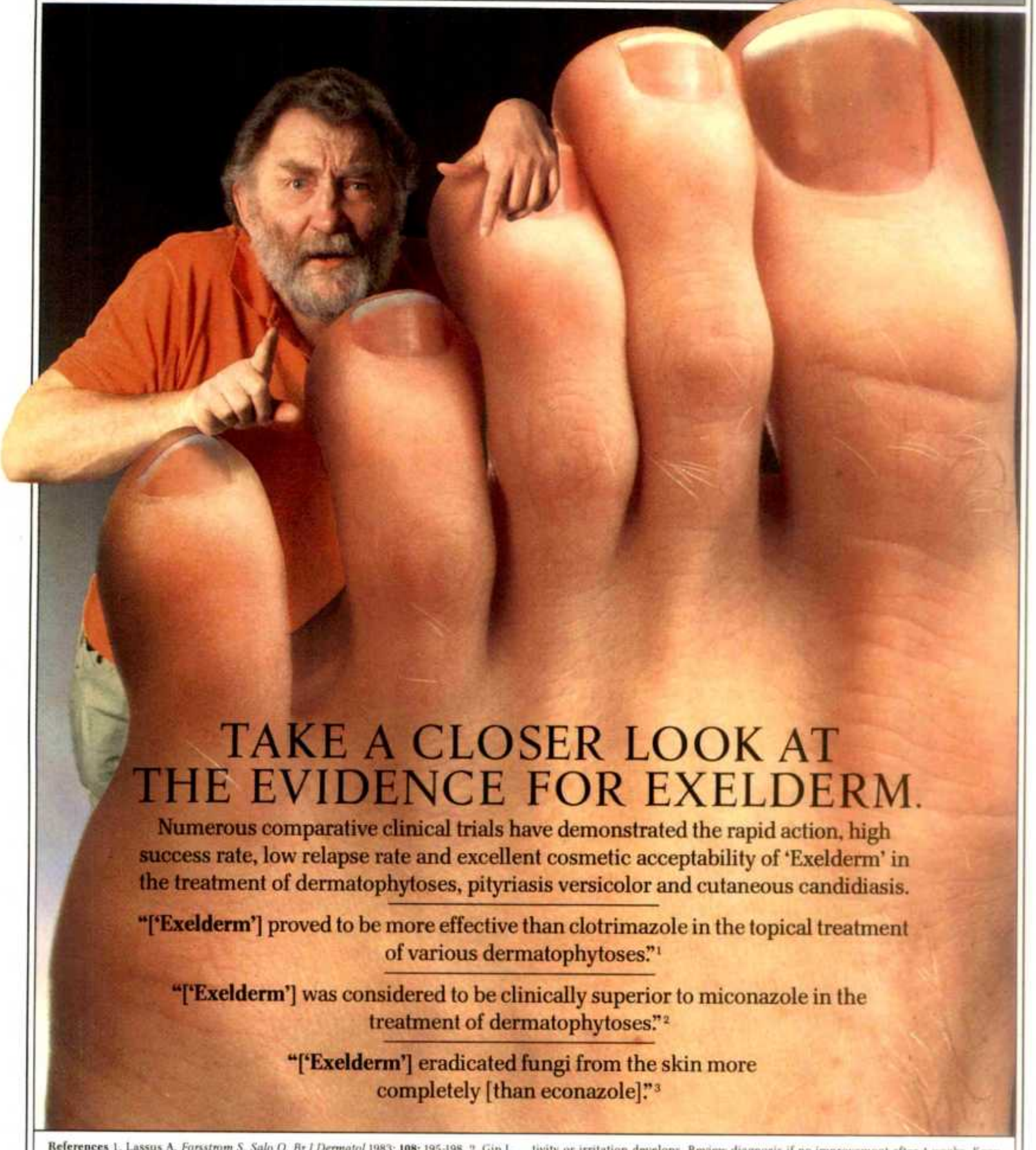

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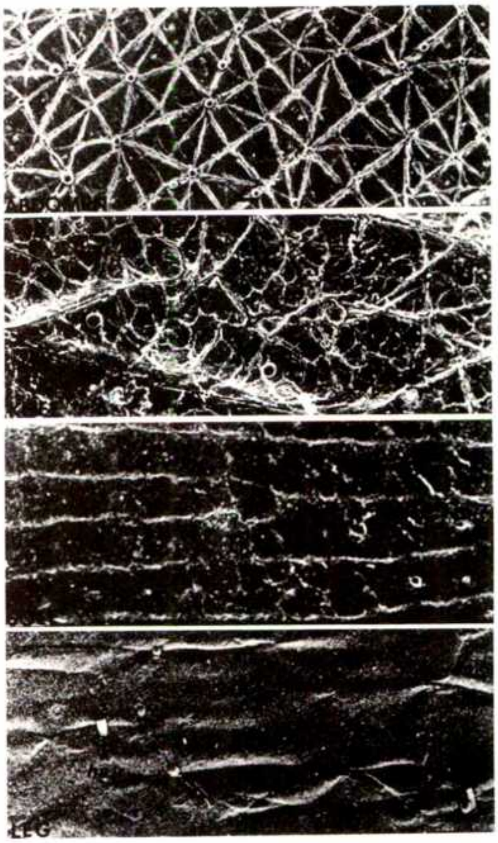

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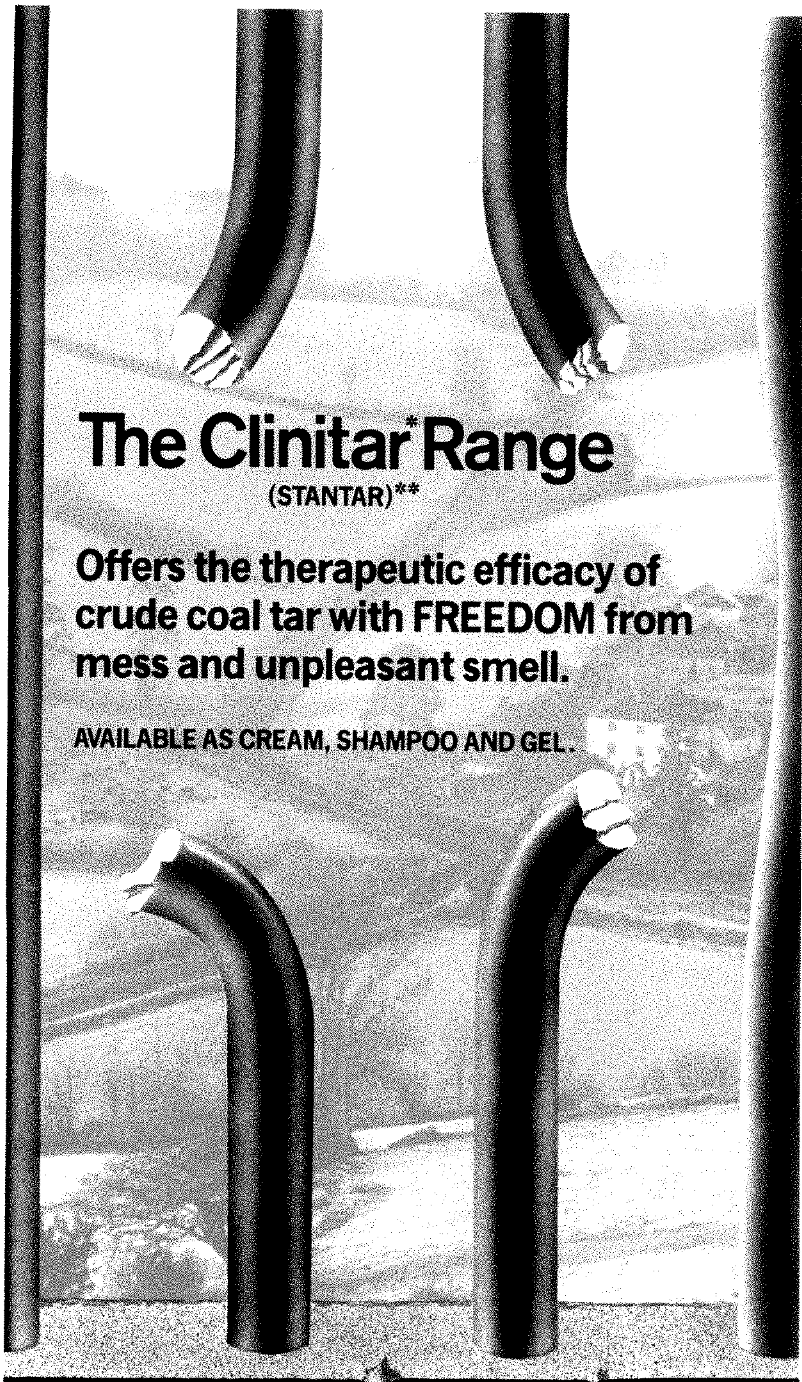

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**LEGAL CATEGORY** P

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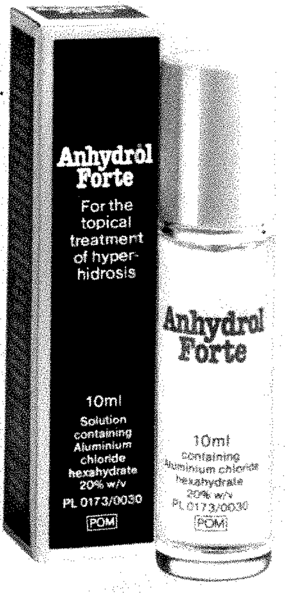
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***Invited Editorial***

# The electron microscope and the antibody: trends in the diagnosis of epidermolysis bullosa

CHRISTINE J.SKERROW
Department of Dermatology, University of Glasgow, Glasgow G12 8QQ, U.K.

The diagnosis of the congenital forms of epidermolysis bullosa (EB) by electron microscopy (EM) provides examples of both the usefulness and the limitations of EM in clinical dermatology. There is a vital need by clinicians and involved families for reliable information: though rare, even the milder forms of these mechanobullous disorders cause physical distress and developmental problems, whilst the severer forms are mutilating and even life-threatening. Precise differentiation between the forms with autosomal dominant and autosomal recessive inheritance is obviously essential for genetic counselling. However, the classification of EB varieties on clinical grounds is not universally agreed upon, and considerable overlap between physical signs exists, particularly in the early stages of the disease. Family histories are not always available and incomplete penetrance and spontaneous mutation are also possibilities.

Electron microscopy has, therefore, come to be the most reliable means of establishing the main, genetically distinct, categories of EB. Numerous studies over the last 20 years (reviewed by Eady & Tidman, 1983, and Kero & Niemi, 1986) have shown that these categories can be identified by differences in the plane of separation within the basement membrane zone (BMZ), and in some cases by qualititative or quantitative modifications to BMZ components such as hemidesmosomes and anchoring fibrils (AF). The ultrastructural classification has been refined further by more recent applications of morphometric analysis (Tidman & Eady, 1985). These studies have exposed an area of uncertainty in the diagnosis of dystrophic EB (DEB), involving two variants with different modes of inheritance. One subgroup of recessive DEB (RDEB) which is relatively mild and localized has been shown to be clinically and ultrastructurally similar to typical dominant DEB (DDEB). Morphometric analysis extended this similarity to the distribution of AF, which were reduced in both cases. AF were, as previously reported, absent in the more severe RDEB form; however, the nature of AF and the sampling errors inherent in EM make even this distinction difficult to make. AF have no consistent ultrastructural features and their distribution even within a single biopsy is extremely heterogeneous (Tidman & Eady, 1984). Secondary degradation of AF following blister formation can also occur. As a result, even in an ideal biopsy the confirmation of total absence of AF (severe generalized RDEB) as opposed to their sparse occurrence (localized RDEB) may require the examination of considerable lengths of the dermal-epidermal junction.

In the dystrophic category of EB, then, we have one distinction which it is technically difficult for EM to make, and another to which EM makes no contribution. For this reason, recent reports of monoclonal antibodies directed towards BMZ components involved in the dystrophic EB group have been particularly welcome. KF-1, which recognizes a non-collagenous component of the basal lamina, reveals reduced staining relative to normal tissue and other forms of EB in the dystrophic group, particularly marked in RDEB (Fine *et al.*, 1984). AF1 and AF2, which react with AF, discriminate more sharply, staining being absent in generalized RDEB and intense in DDEB (Goldsmith & Briggaman, 1983). Neither antibody has been reported to distinguish between localized RDEB and DDEB.

In this issue, there is a report on an antibody, LH 7:2, which reacts with the basal lamina of stratified epithelia (Heagerty *et al.*, 1986, p. 125). LH 7:2 gives distinctive patterns of staining of the various DEB types, including for the first time discrimination between localized RDEB and DDEB. It has also been used successfully in prenatal diagnosis. The application of antibodies such as LH 7:2 will clearly be of benefit both to clinical dermatology and to basic research on the pathogenesis of EB. So far, however, these applications are at an early stage and based on relatively small numbers of patients. EM is likely to remain the major confirmatory technique in EB diagnosis for some time to come, but supplementation of ultrastructural studies by immunohistochemical data, especially in specific problem areas, will undoubtedly increase. This balance may well eventually reverse, as existing antibodies become more well-defined and widely available, and as antibodies to other BMZ components are sought and characterized.

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*British Journal of Dermatology* (1986) **115**, 125–131.

***Laboratory and Clinical Investigations***

# Identification of an epidermal basement membrane defect in recessive forms of dystrophic epidermolysis bullosa by LH 7:2 monoclonal antibody: use in diagnosis

A.H.M.HEAGERTY, A.R.KENNEDY, I.M.LEIGH*, P.PURKIS*
AND R.A.J.EADY

Department of Electron Microscopy and Cell Pathology, The Institute of Dermatology, Homerton Grove, London E.9
and * Laboratory of Experimental Dermatology, The London Hospital, London E1, U.K.

Accepted for publication 22 April 1986

## SUMMARY

LH 7:2 is a monoclonal antibody that was raised against an extract of human epidermal cells and identifies an epitope within the lamina densa of the basement membrane of stratified squamous epithelia. Using indirect immunofluorescence we found intense labelling with LH 7:2 at the epidermal basement membrane (EBM) of normal skin, and in skin samples from patients with simplex, junctional, dominantly inherited dystrophic and acquired forms of epidermolysis bullosa (EB), as well as bullous pemphigoid. Staining was absent or only very faint in generalized recessive dystrophic EB (RDEB), and patchily reduced in the localized form of RDEB.

We conclude that LH 7:2 recognizes an EBM antigen which may be important in the pathogenesis of RDEB. Moreover, the antibody provides a useful probe for the rapid diagnosis of RDEB and is of special value in helping to discriminate between localized RDEB and typical dominant dystrophic EB—conditions which closely resemble each other clinically and which cannot be distinguished by means of transmission electron microscopy.

Diagnosis of the dystrophic forms of epidermolysis bullosa (DEB) is dependent on the presence of trauma-induced blistering, scar formation, milia and nail dystrophy. Confirmation of the diagnosis rests on the demonstration of a split beneath the lamina densa of the epidermal basement membrane (EBM) by electron microscopy (TEM) (Pearson, 1962; Eady & Tidman, 1983), or by antigen mapping using indirect immunofluorescence (IIF) (Hintner *et al.*, 1981). In

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This work was presented in part at the Annual Meeting of the European Society for Dermatological Research, Amsterdam, in May 1985.

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its fully expressed state, generalized recessive dystrophic EB (RDEB) can usually be distinguished from other forms of EB on purely clinical grounds (Gedde-Dahl, 1971). In addition, TEM shows a lack of normal anchoring fibrils in non-lesional skin (Briggaman & Wheeler, 1975; Tidman & Eady, 1985). However, in the absence of a family history, it is frequently impossible to make a clinical distinction between the milder or localized form of RDEB and dominant DEB (DDEB) because the two conditions may be very similar. Further, ultrastructural morphometry of anchoring fibrils fails to discriminate between the two disorders, there being reduced numbers of anchoring fibrils in both (Tidman & Eady, 1985).

We describe here the use of a novel murine monoclonal antibody, LH 7:2, which was raised against a 1% Nonidet P40 extract of human keratinocytes (Leigh & Purkis, 1985) and which identifies an abnormality of the EBM in both generalized and localized RDEB. LH 7:2 can be used as a diagnostic probe for these forms of DEB and is therefore of special value in genetic counselling. In normal tissues, binding is confined to basement membranes of stratified squamous epithelia such as skin, oesophagus and cervix, but not of simple epithelia such as stomach and kidney (Leigh & Purkis, 1985). Preliminary characterization of LH 7:2 has excluded binding to laminin, entactin, fibronectin and collagen types I–VI (Leigh & Purkis, 1985).

## METHODS

### *Subjects and samples*

Ellipse and shave skin biopsies of unblistered skin were obtained from the following:

(a) Fifteen patients with generalized RDEB aged from 9 months to 37 years (Table 1). All parents were unaffected, and only four patients (Patients 4, 6, 9 and 12) had affected siblings, confirming the recessive mode of inheritance. Widespread blistering, erosions and dystrophic scars were found in all patients. Lesions were present at sites of friction and trauma, particularly over bony prominences. Nails and teeth, where present, were dystrophic. Multiple milia and some degree of pseudosyndactyly were evident in all but the youngest patients. Skin biopsies were taken from various sites as shown in Table 1. In Patient 15, samples were obtained from four sites to permit a regional study.

(b) Four patients with verified family histories of localized RDEB (Table 2), that is with at least one affected sibling but unaffected parents. Lesions in these patients and their siblings tended to be limited to bony prominences, hands and feet. In addition, there was milia formation and nail dystrophy, but no pseudosyndactyly. TEM had previously demonstrated structurally normal but reduced numbers of anchoring fibrils. Two further patients, Patients 20 and 21, a brother and sister with localized disease but with early webbing and flexion contractures of the fingers, and some dysphagia, were also examined; these have been reported elsewhere as possessing attenuated anchoring fibrils (Nanchahal & Tidman, 1985).

(c) Eight patients with documented family histories of DDEB, over at least two generations.

(d) A non-DEB group, including three patients with the lethal (Herlitz) variant of junctional epidermolysis bullosa (JEB), three patients with the non-lethal variant of JEB, two patients with epidermolysis bullosa simplex (EBS), and three obligate heterozygotes for severe RDEB. In addition, perilesional biopsies were taken from two patients with bullous pemphigoid (BP) and three patients with epidermolysis bullosa acquisita (EBA). Thirty normal skin specimens were also obtained from healthy adult volunteers and surgical amputations.

Skin samples were examined by TEM using standard techniques (Tidman & Eady, 1984) or antigen mapping after the methods of Hintner *et al.* (1981) in order to establish the level of

TABLE 1. Generalized recessive dystrophic epidermolysis bullosa: patient details and LH 7:2 labelling characteristics

| Patient | Age (years) | Sex | Oesophageal involvement/ dysphagia | Sub-lamina densa split | Anchoring fibrils | Biopsy site | LH 7:2 labelling |
|---|---|---|---|---|---|---|---|
| 1 | 4 | M | + | + | – | Lower leg | – |
| 2 | 37 | F | + | + | – | Thigh | – |
| 3 | 6 | F | + | + | – | Thigh | – |
| 4 | 22 | M | + | + | – | Lower leg | – |
| 5 | 15 | M | + | + | – | Upper arm | Faint |
| 6 | 24 | M | + | + | – | Upper arm | – |
| 7 | 36 | F | + | + | ND* | Thigh | – |
| 8 | 15 | F | + | + | ND | Abdomen | – |
| 9 | 32 | M | + | + | – | Thigh | – |
| 10 | 16 | F | + | + | – | Thigh | Patchy |
| 11 | 4 | M | + | + | – | Lower leg | – |
| 12** | 9 months | F | – | + | – | Buttock | – |
| 13 | 24 | F | – | + | – | Forearm | Patchy |
| 14 | 17 | M | + | + | – | Hand | – |
| 15 | 24 | F | + | + | ND* | Upper arm<br>Abdomen<br>Thigh<br>Lower leg | – |

–, Absent.

+, Present.

* Unable to assess anchoring fibrils because of dermo-epidermal separation in biopsy samples.

** Pseudosyndactyly was absent.

blistering. Many patients had been included in a previous report of anchoring fibril analysis (Tidman & Eady, 1985).

### *Antibodies*

A murine supernatant monoclonal antibody preparation LH 7:2, raised against 1% Nonidet P40 extract of trypsinized cultured keratinocytes, prepared as described previously (Leigh & Purkis, 1985), was used in this study. A rabbit polyclonal antiserum raised against type IV collagen derived from human placenta (kindly provided by Dr V.C.Duance, Food Institute, Langford, U.K.), was used at a titre of 1/600 to confirm the presence of an intact lamina densa.

Rabbit anti-mouse IgG (Dako Ltd, High Wycombe, Buckinghamshire, U.K.) and goat anti-rabbit IgG (Miles Laboratories, Stoke Poges, Slough, U.K.) fluorescein isothiocyanate (FITC) conjugates were used at titres of 1/100 and 1/50 respectively, after pre-absorbing against acetone extracted human skin powder (25 mg/ml).

### *Indirect immunofluorescence studies*

Skin samples were embedded in OCT compound (Lab-Tek Products, Naperville, Illinois, U.S.A.) oriented on cork, snap frozen, and stored at –70°C until required. LH 7:2 antibody (undiluted) and anti-type IV collagen antiserum were incubated with 4 μm serial cryostat sections for 30 min at 37°C, and then washed in excess phosphate buffered saline (PBS). All

TABLE 2. Localized recessive dystrophic epidermolysis bullosa: patient details and LH 7:2 labelling characteristics

| Subject | Age (years) | Sex | Oesophageal involvement/ dysphagia | Sub-lamina densa split | Anchoring fibrils | Biopsy site | LH 7:2 labelling |
|---|---|---|---|---|---|---|---|
| 16 | 12 | M | – | ND | + | Thigh* | Patchy |
| 17 | 61 | F | +† | + | + | Thigh | Patchy |
| 18 | 57 | F | +‡ | + | + | Thigh | Patchy |
| 19 | 38 | M | + | + | +<br>+ | Hand§<br>Forearm* | Patchy |
| 20 | 22 | F | + | + | +¶ | Hand | Patchy |
| 21** | 20 | M | + | + | +¶ | Shoulder§ | Patchy |

–, Absent.
+, Present.
* Non-predilection site.
† Associated with Paterson-Kelly-Brown syndrome.
‡ Associated with hiatus hernia.
§ Predilection site.
¶ Anchoring fibrils attenuated (Nanchahal & Tidman, 1985).
** Early webbing and flexion contracture of fingers.

dilutions were made in PBS, pH 7·4, containing 3% (w/v) bovine serum albumin. Sp2 OAg 14 myeloma cell line supernatant was tested regularly to ensure that inherent antibody formation was absent. Pooled non-immune rabbit serum was used as a further negative control. After incubation with the appropriate FITC-conjugated antiserum, sections were washed in PBS and mounted in glycerol/PBS (9:1 v/v), pH 8·4 containing 0·1% *p*-phenylenediamine as an anti-fade agent (Johnson & Noguira Araujo, 1981) and examined using a Zeiss Standard 14 epi-fluorescence microscope. Photographs were taken using Kodak Ektachrome 200 ASA (slide) film. Results were available within 4 h. End-titres were performed on skin sections from normal subjects and from three patients with the localized form of RDEB.

## RESULTS

In all normal skin samples (Fig. 1a) and in all samples from patients with DDEB (Fig. 1b), JEB, EBS, BP and EBA, staining of epidermal basement membrane was intense, regardless of whether dermo-epidermal separation had or had not occurred. Similarly, labelling was strongly positive in the three obligate heterozygotes.

Normal staining of the EBM with anti-type IV collagen antiserum was present in all 15 patients in the generalized RDEB group. LH 7:2 labelling was absent in 12 of these 15 cases (Fig. 1c; Table 1). In one patient (Patient 5) staining was present but very faint, and in two patients (Patients 10 and 13) patchy reduction was evident. The regional study showed no labelling in any sample from the one patient with generalized RDEB examined. Labelling in the

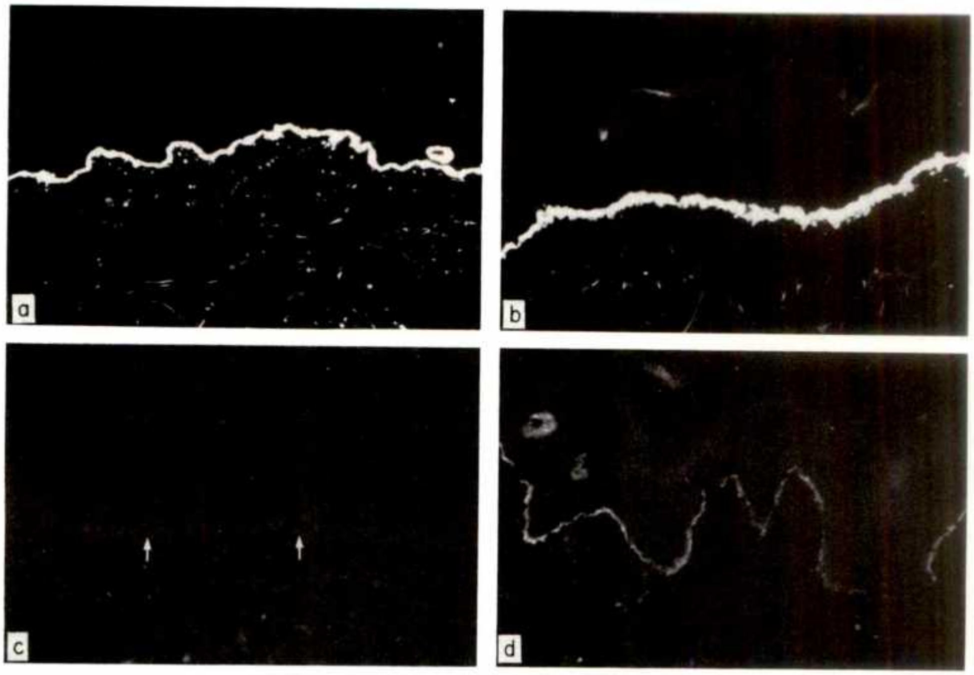

FIGURE 1. Immunofluorescence photomicrographs (original × 190). (a) Intense labelling of epidermal basement membrane (EBM) in normal skin. Note also autofluorescence associated with elastic fibres. (b) Intense labelling of EBM in dominant dystrophic epidermolysis bullosa. (c) Absence of labelling of EBM in generalized dystrophic epidermolysis bullosa. Arrows indicate dermo-epidermal junction. (d) Patchy labelling of EBM in localized dystrophic epidermolysis bullosa.

four cases with localized RDEB displayed a patchy reduction (Fig. 1d; Table 2). End-titres examined in three of these cases were the same as those found in normal skin (1 in 2560). The two siblings with relatively severe but localized RDEB (Patients 20 and 21) also showed patchy labelling. A patchy reduction in staining was evident in both predilection and non-predilection sites in one patient with localized RDEB.

Dermal autofluorescence was seen in all samples from normal controls (Fig. 1a), except in those from very young subjects. It was reduced in the skin of patients with generalized RDEB (Fig. 1c), corresponding with a reduction in elastic fibres identified by staining with orcein.

## DISCUSSION

This study has shown that the monoclonal antibody LH 7:2 provides a valuable new probe for the rapid diagnosis of different forms of RDEB. Skin samples from all patients with verified DDEB displayed intense IIF labelling of the epidermal basement membrane whereas those from all subjects with localized RDEB showed a patchy reduction. LH 7:2 appears, therefore, to be able to discriminate between these two forms of DEB which phenotypically may be very similar and which cannot be distinguished on the basis of ultrastructural criteria (Tidman & Eady, 1985). LH 7:2 is thus of special value in distinguishing patients with DDEB and localized RDEB, in the absence of known involvement of other family members and has an important application in genetic counselling.

In 12 of 15 patients included in the generalized RDEB group, LH 7:2 staining was absent. However, in one case labelling was uniformly faint, and in the remaining two, it was patchily reduced—a pattern more akin to that seen in patients with localized RDEB. These anomalies in staining prompted a reappraisal of the clinical diagnosis of the two patients, Patients 10 and 13 (who are not related and had no affected relatives). Both had some degree of webbing of their fingers. Patient 10 was less severely affected in general whereas the disease was more extensive in Patient 13, who had minimally dystrophic teeth but no dysphagia. No structurally normal anchoring fibrils were seen in skin biopsies of either Patient 10 or Patient 13. Tidman and Eady (1985) have pointed out that morphologically normal anchoring fibrils may be so sparse in localized RDEB that it is possible to see none in relatively long segments of the epidermal basement membrane zone. The patchy LH 7:2 labelling may therefore be accounted for by these two patients representing a more severe expression of localized RDEB, or the milder end of the spectrum of generalized RDEB, emphasizing the overlap in clinical severity that can occur among those patients classified as having either generalized or localized RDEB. Although it is still uncertain whether these variants have the same or different gene defects, Gedde-Dahl (1986) has suggested from pedigree evidence that they may represent different mutations in the same gene locus, and that the clinical variant who carries one of each gene will be clinically intermediate in severity.

The usefulness of LH 7:2 in diagnosis has been particularly evident in one further patient without a family history of EB who presented with clinical features compatible with generalized RDEB, including widespread scarring, milia, dystrophic teeth and nail loss, pseudosyndactyly and dysphagia. However, he was of normal stature and managed a remarkably normal lifestyle at 24 years of age. LH 7:2 labelling was intense; TEM showed a sub-lamina densa split associated with structurally normal anchoring fibrils in unseparated skin. Moreover, suction blister times, as measured on his lower abdomen, were within normal limits, pointing away from the diagnosis of generalized RDEB (Tidman & Eady, 1984). These findings suggest that the patient probably represents a new mutation for DDEB and exhibits a relatively severe form of this disease, of which, although rare, there are a number of cases recorded in the literature (Schwartz *et al.*, 1981).

Preliminary immunoelectron microscopic studies have shown that LH 7:2 identifies an epitope within the lamina densa of the EBM (Heagerty *et al.*, 1985). Some labelling occurred just beneath the lamina densa which was not strictly associated with anchoring fibrils. LH 7:2 might be recognizing an epitope related to part of the fibril (now known to contain type VII collagen, Bentz *et al.*, 1983) within the lamina densa. However, we currently have no evidence that the antibody binds to the banded portion of anchoring fibrils below the lamina densa. A relation between LH 7:2 and anchoring fibrils would support the morphometric data on anchoring fibrils in RDEB, but it would be difficult to reconcile normal LH 7:2 labelling with reduced numbers of anchoring fibrils in DDEB. This may indicate instead that the epitope is anchoring fibril-related rather than part of the actual anchoring fibril molecule. Alternatively, the LH 7:2 antigen might be a portion of the anchoring fibril unaffected by the pathogenetic mechanism in DDEB, but affected in RDEB. We may be better placed to answer these questions when we have completed our studies on the characterization of the LH 7:2 antigen. We are at present studying the relationship between LH 7:2 and type VII collagen.

The monoclonal antibody KF-1 (Fine *et al.*, 1984) also recognizes a binding site within the lamina densa and displays reduced labelling in both DDEB and RDEB. Data comparing DDEB and localized RDEB have not yet been published. Two further monoclonal antibodies AF1 and AF2 (Goldsmith and Briggaman, 1983) both fail to label the EBM in generalized RDEB, but display intense staining in DDEB. Again the staining pattern in the more localized form of RDEB has yet to be reported.

In conclusion, LH 7:2 monoclonal antibody identifies an epitope within the lamina densa which shows distinctive patterns of reduced expression in different forms of RDEB. It has considerable potential as a diagnostic probe by IIF in DEB and has lately been used in the successful rapid prenatal diagnosis of generalized RDEB (Heagerty *et al.*, 1986). Characterization of its antigen should shed important new light on the understanding of the pathogenesis of this group of inherited blistering disorders.

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# Secretion of plasminogen activator inhibitor by cultured human epidermal cells

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## SUMMARY

A conditioned medium from cultured human epidermal cells was observed to inhibit the activity of exogenous urokinase. By reverse fibrin autography after SDS polyacrylamide gel electrophoresis, a plasminogen activator inhibitor was detected with a molecular weight of 46000. Using $M_r$ 33000 [$^{125}$I]-labelled urokinase we observed the formation of an enzyme-ligand complex. The molecular weight of this complex was 79000. These results indicate that cultured human epidermal cells secrete a plasminogen activator inhibitor (urokinase inhibitor) with a molecular weight of 46000.

Plasminogen activators (PA), serine proteinases, are thought to play a role in the regulation of localized proteolysis within the microenvironment of cells (Reich, 1978). Cultured human epidermal cells as well as cultured human fibroblasts synthesize PA (Hashimoto *et al.*, 1983a; Scott *et al.*, 1983). Increases in PA activity in several skin diseases such as psoriasis and pemphigus have been described (Fraki, Briggaman & Lazarus, 1982; Hashimoto *et al.*, 1983a). Proteolytic activity is regulated in part by specific inhibitors. Recently, a wide variety of cells have been reported to secrete or produce PA inhibitors, among them human fibroblasts, bovine endothelial cells, human platelets and human peripheral blood monocytes (Scott *et al.*, 1983; Loskutoff *et al.*, 1983; Erickson, Ginsberg & Loskutoff, 1984; Vassali *et al.*, 1984). These results have led us to investigate whether cultured human epidermal cells secrete PA inhibitor.

## METHODS

### *Cell culture*

Human epidermal cells from normal adult skin were cultured on tissue culture plates coated with acid-extracted rat tendon collagen according to the method of Yuspa *et al.* (1980). A serum-free conditioned medium was prepared as follows: epidermal cells in 35 mm dishes (Falcon, Oxnard, CA, U.S.A.) were washed twice with PBS and once with Dulbecco's Modified Eagle's

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Medium (DME). Cells were then incubated with DME for 24 h. This conditioned medium was removed, centrifuged and stored at −70°C.

### *Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)*

After adding concentrated SDS-PAGE sample buffer, samples (10 $\mu$l) were electrophoresed in 10% polyacrylamide mini-slab gels, using the buffer system of Laemmli (1970). All gels were run under non-reducing conditions.

### *Reverse fibrin autography*

Fibrin-agar indicator films for reverse fibrin autography were prepared according to modification of the method of Erickson, Lawrence & Loskutoff (1984). Briefly, solutions of agarose, plasminogen, thrombin, fibrinogen and urokinase were mixed and poured onto a prewarmed glass plate. Final concentrations were 1·3% agarose, 32 $\mu$g/ml plasminogen, 0·08 IU/ml thrombin, 2·25 mg/ml fibrinogen and 75 mIU/ml urokinase. The films were used immediately. After electrophoresis, the SDS gels were soaked in 2·5% Triton X-100 for 1·5 h to remove SDS, patted dry with a paper towel and applied to the surface of the fibrin-agar indicator film. The indicator film was incubated at 37°C in a moist chamber and photographed at various times. An opaque area on the indicator film corresponded to a lysis-resistant region, indicating the presence of PA inhibitor.

### *[$^{125}$I]-labelling of urokinase*

M$_r$ 33000 urokinase ($1{\cdot}6 \times 10^5$ IU/mg), generously provided by Mochida Pharmacy Co., Tokyo, Japan, was labelled using Iodogen as described by Baker *et al.* (1980), except that free $^{125}$I was removed using minicolumns of Sephadex G-25 (Tuszynski *et al.*, 1980). The specific activity of the [$^{125}$I]-urokinase was $0{\cdot}5$–$0{\cdot}8 \times 10^7$ cpm/$\mu$g.

### *Autoradiography*

Gels were stained with Coomasie Blue, destained, dried and autographed using Kodak X-Omat film and developed following 16–48 h exposure.

### *Fluorogenic assay of inhibitor activity*

Urokinase activity was assayed using glutaryl-Gly-Arg-MCA (Morita *et al.*, 1977). The conditioned medium was concentrated up to 16 times using Minicon-B (Amicon, Danvers, MA, U.S.A.). 100 $\mu$l of conditioned medium was mixed with 10 $\mu$l of urokinase at 50 IU/ml and adjusted to a final volume of 1 ml with 0·1 M Tris-HCl buffer, pH 8·1 after pre-incubation on ice for 15 min. 10 $\mu$l of the fluorogenic substrate at a concentration of 10 mM was added. The mixture was incubated at 37°C for 1 h and the enzyme reaction was stopped by adding 50 $\mu$l of 50% acetic acid. The measurements were carried out with excitation at 380 nm and emission at 440 nm.

## RESULTS

The inhibitory activity of the conditioned medium from cultured human epidermal cells was assayed by adding a constant amount of urokinase to medium concentrated up to 16-fold (Fig. 1). When mixed with non-concentrated to 4-fold concentrated medium, urokinase activity decreased in a concentration-dependent manner. When added to medium concentrated 8 or 16-fold, less than 10% of urokinase activity remained. The conditioned medium alone showed no

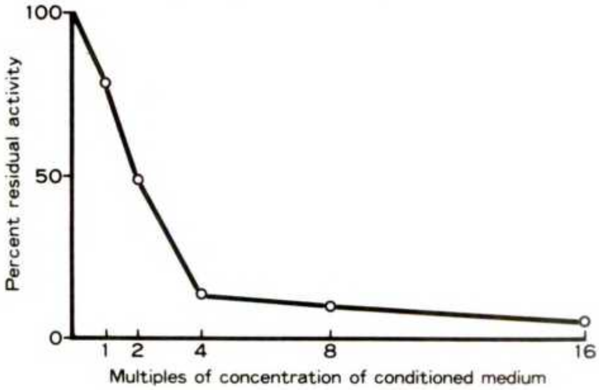

FIGURE 1. Inhibition of urokinase activity by a conditioned medium from human epidermal cells. Inhibitor activity is expressed as a percentage of the control activity.

detectable urokinase activity when assayed using the fluorogenic substrate. This suggested that the cultured human epidermal cells released a PA inhibitor of urokinase.

The conditioned medium from cultured human epidermal cells was subjected to SDS-PAGE and reverse fibrin autography. PA inhibitor was detected as an opaque, lysis-resistant band of $M_r$ approximately 46000 ± 2000 (Fig. 2).

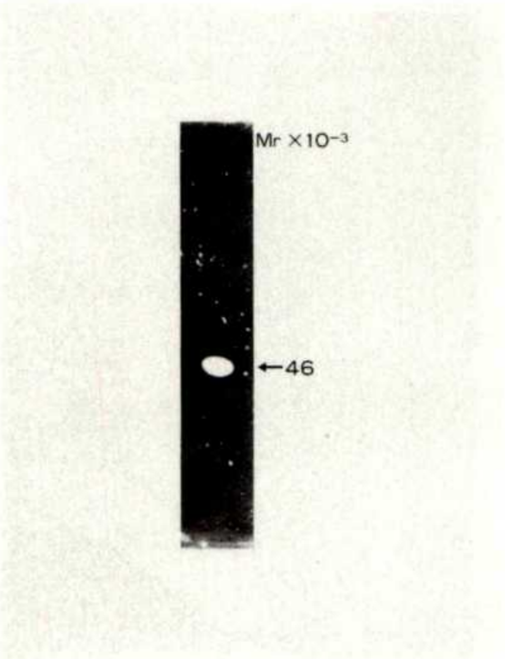

FIGURE 2. Detection of PA inhibitor in a conditioned medium from human epidermal cells by reverse fibrin autography. The indicator gel was photographed after 6 h incubation. Molecular weight was calculated from the position of markers (Bio-Rad, low molecular weight standards) electrophoresed in parallel lanes and stained with Coomasie Brilliant Blue R 250.

When $M_r$ 33000 [$^{125}$I]-urokinase was added to concentrated medium from cultured human epidermal cells, autoradiography following SDS-PAGE showed that all the radioactivity migrated in a band with an $M_r$ of 79000 ± 3000 (Fig. 3a, lane 2). When non-concentrated medium was mixed with [$^{125}$I]-urokinase, only a small portion of the radioactivity migrated. In some experiments using different batches of conditioned medium, the radioactive band appeared as a doublet with a lower band of $M_r$ 68000 ± 3000 (Fig. 3b). This suggests that [$^{125}$I]-urokinase had formed a complex with PA inhibitor in the medium. Taken together, these results demonstrate that cultured human epidermal cells secreted PA inhibitor (urokinase inhibitor) with a molecular weight of 46000 ± 2000.

## DISCUSSION

Production and secretion of PA inhibitors have been described in a variety of cells. In bovine endothelial cells a PA inhibitor of $M_r$ 50000 is synthesized and secreted. This inhibitor is acid-stable, and inhibits both urokinase and tissue PA (Loskutoff *et al.*, 1983; van Mourik, Lawrence & Loskutoff, 1984). In contrast, protease nexin of $M_r$ 51000, a PA inhibitor produced by human fibroblasts, is acid-labile. It is an effective inhibitor of urokinase but not a good inhibitor of tissue PA (Scott & Baker, 1983). Human cells of monocyte-macrophage lineage also produce a PA inhibitor of $M_r$ 40000. This inhibitor is different from protease nexin because it cannot interact with thrombin (Vassali *et al.*, 1984). These inhibitors appear to differ from each other. However, Erikson, Ginsberg & Loskutoff (1984) demonstrated that PA inhibitor in human platelets was immunologically similar to PA inhibitor in bovine endothelial cells. This

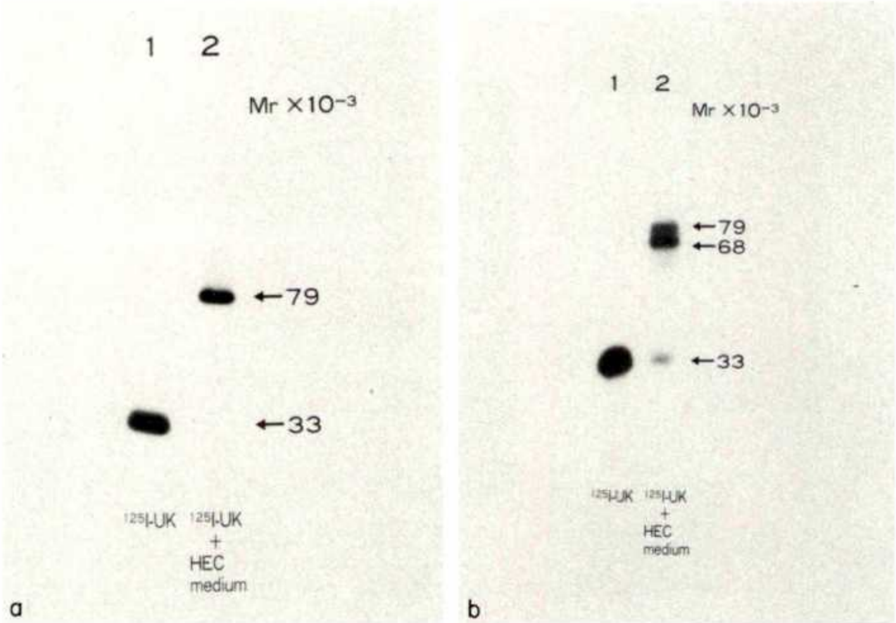

FIGURE 3(a and b). Autoradiography of mixtures of [$^{125}$I]-urokinase and conditioned medium from two different cultures of human epidermal cells (HEC). Mr 33000 [$^{125}$I]-urokinase (1 ng) was added to 20 $\mu$l of DME (lane 1) and of a 16-fold concentrated conditioned medium (lane 2). Molecular weights were calculated from the position of markers in the stained gels.

suggested the possibility that cellular PA inhibitors can be classified into several groups immunologically. We have described here the secretion of PA inhibitor by cultured human epidermal cells. However, we cannot discuss the relationship of our inhibitor to those outlined above since we have not yet defined its antigenicity compared with other inhibitors, its inhibitor specificity to tissue PA, its interaction with thrombin nor its stability under acid treatment. In the epidermis, Nishioka and Ryan (1971) reported the presence of urokinase inhibitor. We have reported the presence of tissue PA inhibitor as well as urokinase inhibitor in the epidermis (Hashimoto, Katayama & Nishioka, 1985). The present data suggests the possibility that these epidermal PA inhibitors are endogenous. Cultured human epidermal cells are known to synthesize and secrete PA (Hashimoto *et al.*, 1983a). The concomitant secretion of PA and of PA inhibitor has been reported for cultured human fibroblasts and human monocytes-macrophages (Scott *et al.*, 1983; Vassali *et al.*, 1984). These paradoxical findings are explained by the observation that urokinase PA is present as a urokinase proenzyme which is activated by plasmin. We also observed that urokinase PA was present as a proenzyme in a medium from cultured human epidermal cells (unpublished data). Our findings support the suggestion that this is a general phenomenon.

It is noteable that a complex of [$^{125}$I]-urokinase and PA inhibitor appeared as a doublet in some batches of conditioned medium from human epidermal cells. The cause of the doublet has not been determined. The lower molecular weight band may represent degraded products of PA inhibitor or the two bands may reflect heterogeneity of PA inhibitors. Physiological and pathological roles of PA inhibitor in cultured human epidermal cells remain conjectural. Recent studies have demonstrated an increase in PA activity in several skin diseases such as psoriasis and pemphigus. PA inhibitor may play a significant role in these diseases through the regulation of PA activity.

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# Contraction of collagen lattices by fibroblasts from patients and animals with heritable disorders of connective tissue

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## SUMMARY

Fibroblasts derived from patients and animals presenting various heritable connective tissue disorders were investigated for the ability to retract a reconstituted collagen matrix. When seeded into gels, dermatosparactic calf and sheep fibroblasts did not exhibit the elongated shape of normal fibroblasts and did not contract the collagen lattice to the same extent as control fibroblasts. In contrast, several cell strains obtained from patients with Ehlers-Danlos syndrome type VII displayed contractile properties for collagen gels similar to controls. Delayed contraction was noted by two strains of fibroblasts from patients with Ehlers-Danlos syndrome type IV, whereas fibroblasts from patients with osteogenesis imperfecta, Marfan syndrome and cutis laxa had normal retraction properties.

The major structural component of the extracellular matrix is collagen. This protein, known to be present as different molecular species, undergoes several post-translational enzymatically controlled modifications (Sage & Bornstein, 1980). Inborn disorders involving one of these reactions have been reported by many workers (for review *see* Prockop & Kivirikko, 1984).

Although the phenotypic expression of these collagen disorders is markedly heterogeneous, involvement of the skin, manifested as a modification of its mechanical properties, is a common feature. The clinical symptoms are most pronounced in the different types of the Ehlers-Danlos syndrome (Ihme *et al.*, 1981) and in some animal models including dermatosparaxis in cattle (Lenaers *et al.*, 1971) and sheep (Fjolstad & Helle, 1974).

Defective synthesis and processing of type III collagen have been reported in patients suffering from Ehlers-Danlos syndrome (EDS) type IV (Pope *et al.*, 1975; Aumailley *et al.*, 1980; Byers *et al.*, 1979). A mutation of lysyl-hydroxylase has been shown to be present in EDS

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type VI (Pinnell *et al.*, 1972). Conversion of procollagen is defective in dermatosparaxis (Lenaers *et al.*, 1971) and also in patients with the recessive form of EDS type VII (Steinmann *et al.*, 1980). In patients with osteogenesis imperfecta (OI) types I and IV, an altered ratio of collagen types I and III has been described (Penttinen *et al.*, 1975), whereas in OI type II, an increased hydroxylation of lysine residues has been observed (Trelstad, Rubin & Gross, 1977; Kirsch *et al.*, 1981). Both alterations are probably indirectly related to mutations in the collagen type I gene (Williams & Prockop, 1983; de Wet *et al.*, 1983; for review *see* Prockop & Kivirikko, 1984).

Recently, the culture of fibroblasts in a collagen gel has been developed to mimic cell matrix interactions *in vitro* (Bell, Ivarsson & Merrill, 1979; Yasui *et al.*, 1982). Skin fibroblasts grown under these conditions attach rapidly to the fibres and contract the gel. This ability is thought to represent a physiological property of fibroblasts which is also expressed by myofibroblasts in the contraction of wounds. It has been shown recently that dermatosparactic (D) calf fibroblasts lack the capacity to retract a collagen lattice (Delvoye, Nusgens & Lapiere, 1983). We report here the results of further studies using D-sheep, and a comparison with the human counterpart of this disease, EDS type VII. In addition, a large number of fibroblasts from other inborn disorders of connective tissue have been tested using the same model.

## METHODS

Biopsies were taken from patients with inborn disorders of connective tissue metabolism under local anaesthesia. Fibroblasts were obtained from explant cultures as described earlier (Ihme *et al.*, 1983).

### *Patients*

Patients with osteogenesis imperfecta were diagnosed and classified according to Sillence, Senn and Danks (1979). The detailed clinical description of the patients from which fibroblast cultures were established are presented elsewhere (Krieg *et al.*, 1981). Experiments were carried out with fibroblasts obtained from patients with osteogenesis imperfecta types II, III and IV at between passage 4 and 15.

Patients with Ehlers-Danlos syndrome were classified as types I, II, IV and VI according to the clinical symptoms and genetic and biochemical criteria described earlier (Ihme *et al.*, 1981). Three strains of fibroblast (CLR 1183, 1150, 1148) from patients with EDS type VII were obtained from the American type culture collection.

Patients with the autosomal dominant form of cutis laxa and pseudoxanthoma elasticum as well as patients with Marfan syndrome were characterized clinically according to McKusick (1972). The patient with progeria has been described earlier (Rautenstrauch *et al.*, 1977). The fibroblast cultures were used between passages 2 and 7.

Fibroblast cultures were established from skin biopsies from sheep and calves with dermatosparaxis and control, unaffected, animals, and used in passages 4 to 8 (Wiestner *et al.*, 1982; Delvoye *et al.*, 1983).

Fibroblast cultures were also established from skin biopsies from patients of different ages with diseases unrelated to connective tissue disorders.

### *Cell culture in collagen gels*

Collagen type I was extracted and sterilized as described previously (Lapiere, Nusgens & Pierard, 1977), and used at a concentration of 1 mg/ml in dilute acetic acid (1:1000).

The cells were treated briefly with 0·25% trypsin, removed from the culture dish and suspended in 5 ml of DMEM supplemented with 10% foetal calf serum (FCS). The cells were counted on a Thoma's plate, centrifuged for 8 min at 1200 *g* and diluted to the desired concentration in DMEM with 10% FCS.

The lattices were prepared in Petri dishes using the technique described earlier (Delvoye *et al.*, 1983). Briefly, 1·85 ml of a 2·5 × concentrated medium was added to 1·5 ml of collagen solution (1 mg/ml) and the suspension of cells in 0·5 ml of DMEM. This mixture polymerized in less than 1 min at 37°C. After 2 and 4 days, 2 ml of medium were removed, and an equivalent volume of fresh DMEM containing 10% FCS was added. The diameter of the lattice was measured every day.

Cell viability and growth were measured by Neutral Red staining.

## RESULTS

Normal human skin fibroblasts seeded at different densities contracted the collagen lattice at a rate proportional to the cell number (Fig. 1). There was no apparent dependence on age (Fig. 2) or sex, or on the number of passages (from 6 to 27). This was supported further by the observation of a normal contraction of collagen gels by fibroblasts derived from a patient with progeria (not illustrated). All cells reduced the size of the lattice within 24 h down to 20% – 10% of the initial diameter. Contraction was complete after 4 days and did not change further.

Calf and sheep dermatosparactic fibroblasts in monolayers differed in shape when compared to controls and had an epitheloid shape at confluency (Lozano, Helle & Muller, 1983). In contrast, normal calf or sheep fibroblasts displayed the typical elongated shape found in the normal human skin fibroblasts. Whereas normal calf and sheep fibroblasts aligned along the fibres in the collagen gel and contracted the lattice at a rate similar to normal human fibroblasts, dermatosparactic sheep cells did not attach to the polymers, and displayed a delayed and reduced capacity for contraction of the lattice (Fig. 3).

When fibroblasts from patients with disorders of collagen metabolism were seeded into a collagen gel, all the cells displayed the classical elongated shape and organized around them bundles of collagen fibres in a radial configuration. All fibroblasts derived from the different EDS patients, including EDS VII, contracted the collagen gels to an extent similar to that in the controls (Table 1). However, with fibroblasts from two patients with EDS type IV, the retraction of the gels was delayed. Results with fibroblasts from the patients with other inborn connective tissue disorders did not differ from the controls (Table 2).

## DISCUSSION

The contraction of a collagen lattice by fibroblasts in culture results from a specific interaction between the cells and the collagen fibres. We have reported previously that the capacity for retracting a collagen gel is reduced in fibroblasts from D-cattle (Delvoye *et al.*, 1983), which have a disorder of collagen metabolism characterized by defective activity of pN-collagen peptidase (Lapiere, Lenaers & Kohn, 1971; Lenaers *et al.*, 1971).

In D- sheep, where the processing of procollagen type I is defective (Wiestner *et al.*, 1982), the capacity for retracting the gel was noticeably reduced. Fibroblasts from patients with EDS type VII were used in order to elucidate whether defective cleavage of pN-peptide is implicated in the lack of ability of D-cells from both animal species to contract a collagen gel. In one cell strain derived from a patient with EDS type VII (ATCC, CRL 1183), pN-protease activity has been reported to be normal and a mutation at or near the cleavage site is responsible for the

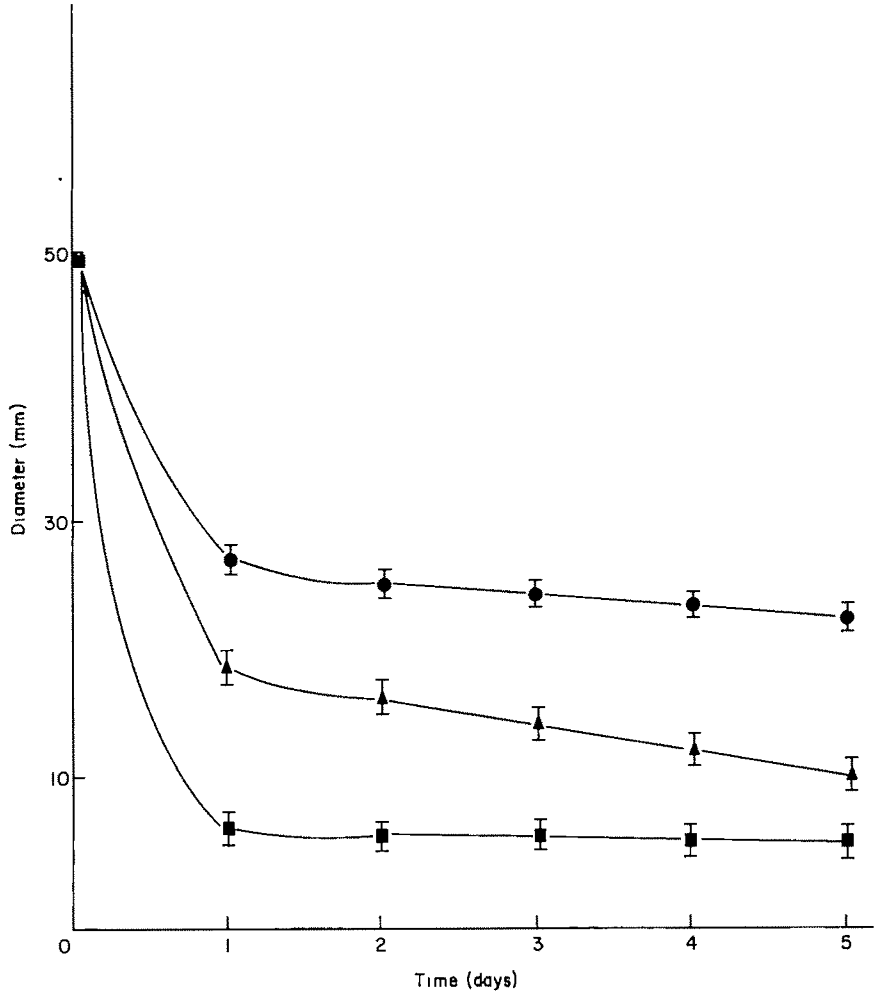

FIGURE 1. Retraction of a collagen lattice by normal human skin fibroblasts. The lattice contained 0·3 mg/ml collagen. Points represent mean and range of duplicate cultures. ● = 1 × $10^5$ cells; ▲ = 2 × $10^5$ cells; ■ = 3 × $10^5$ cells.

accumulation of pN-alpha 2-peptide (Steinmann *et al.*, 1980). In CRL 1150 pN-protease activity is reduced. The alteration responsible for the defect in the strain CRL 1148 from a patient with EDS VII is not known. However, all strains of EDS type VII contracted the gel normally, which indicates that the lack of activity of pN-peptidase or the altered processing of the procollagen molecule is not sufficient to explain the defective contraction by fibroblasts from D-calves and sheep.

Fibroblasts from EDS type IV have been found to exhibit a marked reduction in synthesis of type III collagen (Aumailley *et al.*, 1980). A delay in collagen lattice contraction was noted with these fibroblasts. In contrast, fibroblasts obtained from a patient with osteogenesis imperfecta type IV, exhibiting a decreased ratio of type III: type I collagen (Krieg *et al.*, 1981) which is probably due to a decreased synthesis or secretion of type III collagen, and all other cell strains

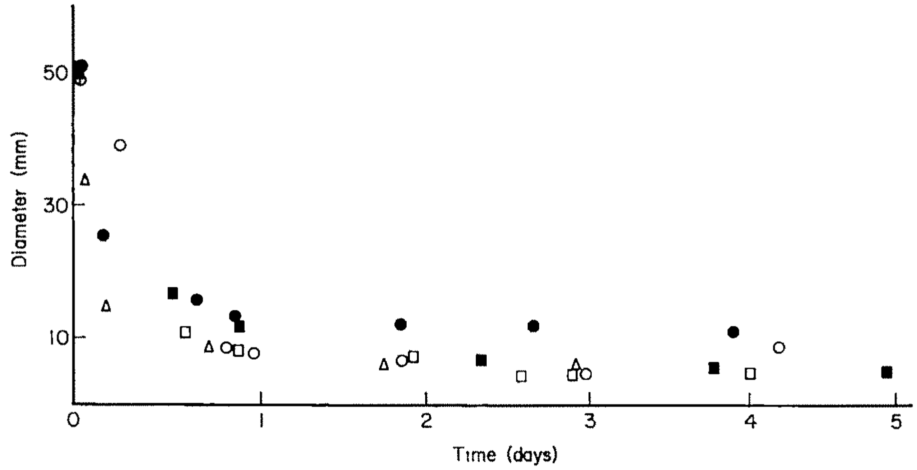

FIGURE 2. Collagen lattice contraction by normal human skin fibroblasts in relation to the age of the subject. ● = embryo; ○ = 10 years; ■ = 25 years; □ = 33 years; △ = 84 years. Results presented for cells between passages 2 and 7. Similar results were observed at passages 15 to 20 and 24 to 27.

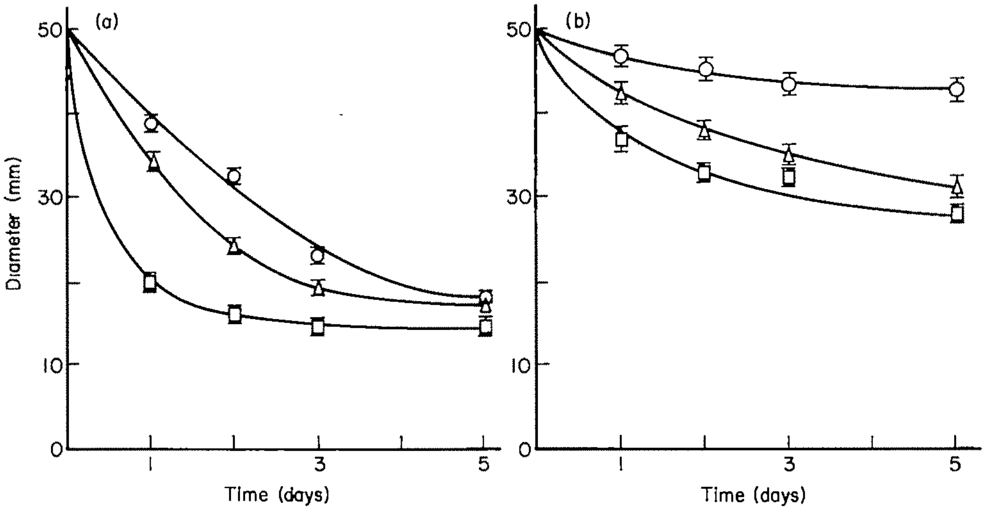

FIGURE 3. Contraction of collagen latices by fibroblasts from (a) normal sheep and (b) D-sheep. ○ = $1 \times 10^5$ cells; △ = $2 \times 10^5$ cells; □ = $3 \times 10^5$ cells. Points represent mean and range of duplicate cultures.

contracted collagen gels similarly to the controls. This indicates that the relative amounts of collagen types I and III synthesized does not affect this process. In addition, since several of the patients investigated suffered from disturbed wound healing, contraction of collagen gels *in vitro* probably reflects only some aspects of the contraction of wounds.

Cell shape in the gel matrix is obviously related to the capacity for contracting the gel. This has also been suggested by the defective behaviour of fibroblasts from the recessive form of epidermolysis bullosa dystrophica in a collagen lattice (Ehrlich *et al.*, 1983). All the cells that

TABLE 1. Contraction of collagen gels by fibroblasts obtained from patients with Ehlers-Danlos syndrome (EDS)

| EDS type | Patient no. | Contraction (mm) after 1 day | Contraction (mm) after 4 days |
|---|---|---|---|
| I | 1 | 10 | 5 |
| | 2 | 11 | 8 |
| | 3 | 9 | 5 |
| II | 4 | 9 | 4 |
| | 5 | 15 | 13 |
| IV | 6 | 23 | 10 |
| | 7 | 18 | 15 |
| VI | 8 | 10 | 8 |
| | 9 | 8 | 7 |
| VII | 10 | 19 | 12 |
| | 11 | 20 | 13 |
| | 12 | 18 | 10 |
| Controls | | 11·8 ± 3·9* | 7·8 ± 3·8* |

* Mean ± SD for four normal controls.

TABLE 2. Contraction of collagen lattices by fibroblasts from patients with heritable connective tissue disorders

| Disorder | No. of cell strains tested | Contraction (mm) after 1 day | Contraction (mm) after 4 days |
|---|---|---|---|
| Osteogenesis imperfecta | | | |
| type II | 3 | 15 | 8 |
| type III | 1 | 7 | 5 |
| type IV | 1 | 8 | 4 |
| Cutix laxa | 2 | 14 | 8 |
| Marfan syndrome | 2 | 10 | 5 |
| Pseudoxanthoma elasticum | 4 | 15 | 9 |
| Controls | 4 | 11·8 ± 3·9* | 7·8 ± 3·8* |

* Mean ± SD.

were able to retract the matrix spread along the collagen fibres and organized the network of fibrils, while cell lines that lacked the property to contract the gel remained rounded or displayed very short extensions. This interaction between cells and extracellular matrix seems to be required to develop and organize the active retraction of the extracellular matrix. The defective retractile properties could be related to the architectural organization of the collagen fibres and bundles of fibres. These are altered in D-animals while no such change has been reported in

EDS type VII. This supports our previous proposal that fibroblasts are involved in the packing of the collagen polymers in the dermis.

Fibronectin, other glycoproteins and membrane receptors are obviously involved in the mechanism of the retraction and study of these should assist interpretation of these observations.

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# Elevated thymosin alpha 1 levels in Brazilian pemphigus foliaceus

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## SUMMARY

Levels of thymosin alpha 1 in the sera of 37 patients with Brazilian pemphigus foliaceus (BPF) were measured using a competitive binding radioimmunoassay. The values were compared with 19 patients with other forms of pemphigus, 13 relatives of patients with BPF, 18 patients with other dermatological diseases, and 265 normal controls. We found that 27 (73%) of the patients with BPF had thymosin alpha 1 serum levels that were at least two standard deviations above the mean for normal individuals. The mean value for patients with BPF was significantly greater than any other groups studied. The thymosin elevation is similar to alterations seen in certain viral diseases and suggests that BPF is aetiopathogenically distinct from the other forms of pemphigus.

Brazilian pemphigus foliaceus (BPF) is an autoimmune bullous disease that affects approximately 10 000 individuals (Proenca & Castro, 1971). It is endemic to certain regions of South America, where cases occur almost exclusively in the rural population (Castro, Roscoe & Sampaio, 1983). This epidemiology suggests strongly that an infectious agent may be involved, possibly transmitted by an insect vector, but no aetiological agent has yet been demonstrated (Angulo, 1954).

Beutner *et al.* (1968) were the first to demonstrate circulating autoantibodies to keratinocyte surface antigens in the sera of patients with BPF. Titres of these antibodies correlate with the severity of clinical disease. We have shown recently that purified IgG fractions prepared from the sera of patients with BPF are capable of inducing the characteristic epidermal lesions in neonatal Balb/c mice (Roscoe *et al.*, 1984). These findings show that the autoantibodies are essential for induction of cutaneous lesions but the aetiology of the disease remains unclear.

Thymic epithelium produces peptides that play a major role in the maturation of immunocompetent T cells. One of the best characterized is thymosin alpha 1, which is known to increase the number of helper T cells and enhance resistance to bacterial, fungal and viral pathogens (Goldstein *et al.*, 1983). Serum levels of thymosin alpha 1 are increased in viral

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diseases such as the acquired immunodeficiency syndrome (AIDS) (Hersh *et al.*, 1983) but are usually normal or decreased in idiopathic autoimmune diseases (P. Naylor, unpublished data).

The present study was designed to determine whether alterations of thymosin alpha 1 levels occurred in patients with BPF, and if so, would those alterations be similar to those observed in autoimmune diseases or to those observed in infectious diseases.

## METHODS

Venous blood was obtained from five groups: 37 patients with well-characterized BPF (mean age 37 years), 13 blood relatives of patients with BPF (mean age 42 years), 19 patients with other forms of pemphigus (mean age 50 years), 18 patients with other types of cutaneous disease (mean age 36 years), and 265 normal blood donors (mean age 40 years).

All patients with the diagnosis of BPF fulfilled the epidemiological, clinical, histological and immunofluorescent criteria for the diagnosis. The blood relatives were free of clinical disease, and 11 of these 19 individuals lived in endemic areas. All patients with the various forms of pemphigus were receiving systemic steroid therapy (approximately 1 mg/kg body weight/day of prednisone or equivalent). The group with other dermatoses was a very heterogeneous group that included diagnoses such as contact dermatitis, deep fungal diseases, psoriatic arthritis and various others.

### *Radioimmunoassay*

Thymosin alpha 1 levels were measured in coded serum samples by a competitive binding radioimmunoassay, using a method previously described (McClure *et al.*, 1982). Normal values were derived from the 265 normal blood donors. Two standard deviations (SD) from this mean was defined as the upper limit of normal. Statistical analysis was performed using a two sample *t*-test with a Bonferroni multiple testing correction, with an overall significance level of 5%.

### *Immunofluorescence*

Indirect immunofluorescence (IF) was performed as previously described (Beutner & Jordan, 1964), using rat tongue as the tissue substrate.

## RESULTS

### *Thymosin alpha 1 levels*

The thymosin alpha 1 serum levels for the various groups are shown in Table 1. The value in the BPF group was 1388 ± 646 pg/ml (mean ± SD), which is significantly higher than the control value of 664 ± 164 pg/ml. 27 out of 37 BPF patients (73%) had thymosin alpha 1 levels that were greater than 2 SD above the mean for normal controls.

The mean for patients with other forms of pemphigus was 598 ± 490 pg/ml, which is significantly lower than the BPF group, although there is some overlap. Nonetheless, only three of these 19 patients with other forms of pemphigus (16%) had values greater than 2 SD above the mean for normal controls.

The level of serum thymosin alpha 1 in relatives of patients with BPF was 723 ± 321 pg/ml, which is not significantly different from that seen in normal controls, but was significantly less than that of BPF patients. Two individuals had significantly elevated levels ( > 2 SD above the mean for normals).

TABLE 1. Serum levels of thymosin alpha 1 in patients and controls

| Group | No. of Subjects | Serum thymosin alpha 1, pg/ml (mean ± SD) |
|---|---|---|
| Brazilian pemphigus foliaceus (BPF) | 37 | 1388 ± 646 |
| Relatives of BPF patients | 13 | 723 ± 321 |
| Pemphigus—other forms | 19 | 598 ± 490 |
| Other dermatoses | 18 | 794 ± 338 |
| Normal controls | 265 | 664 ± 164 |

The group composed of patients with various other dermatoses had a mean thymosin alpha 1 serum level of 794 ± 338 pg/ml. This value was significantly lower than the BPF group.

### *Pemphigus antibody titres*

Sera of patients with BPF were tested by indirect IF on both rat tongue and mouse thymus. The pattern on rat tongue showed the characteristic intercellular substance (ICS) staining, which is indistinguishable from that seen in other types of pemphigus (foliaceus, erythematosus, vegetans and vulgaris), confirming the findings at the time of diagnosis. The sera of relatives of patients with BPF were also tested. We found that two of the 19 relatives had positive ICS titres, one at 1:80 and one at 1:160. Both of these subjects lived in endemic areas but had no skin lesions. None of the other control sera produced a positive ICS pattern by indirect IF.

## DISCUSSION

Our data indicate that thymosin alpha 1 levels are elevated in the BPF group but not in the group with non-endemic forms of pemphigus. This finding cannot be attributed to age differences alone, although thymosin levels are known to decrease with increasing age in normal children. The possibility of the epidermis itself producing thymosin or a thymosin-like molecule as a by-product of acantholysis seems unlikely; if this were the case, one might have expected thymosin levels to be elevated in the group with non-endemic pemphigus. The group with non-endemic forms of pemphigus also acted as a control for the effect of systemic steroids since the average dose (about 1 mg/kg body weight prednisolone or equivalent/day) was similar in both groups.

In addition, we found that the levels of thymosin alpha 1 were not elevated in the group comprising blood relatives of patients with BPF living in the same environment. It is interesting to note that the only relative living with a BPF patient who had an elevated thymosin level also had positive ICS antibodies. The only other relative who had a positive ICS had normal thymosin levels. We are currently following these two individuals to determine whether we can predict the appearance of disease.

The group with other dermatoses was included to determine whether non-specific cutaneous inflammation has any general effect in increasing thymosin levels. This was not found to be the case, but it was observed that all three patients with deep fungal diseases had significant thymosin elevation.

No relationship was found between thymosin levels and the titre of pemphigus antibodies. However, the only untreated BPF patient had the highest level of thymosin measured in this study, which raises the possibility that oral steroid treatment may mask a relationship.

The possibility that the thymosin alpha 1 elevation is due to immunological damage to T cells should also be considered. Cellular immunity to some viruses appears depressed in BPF, and Guerra, Reis and Guerra (1976) have found a decrease in the total numbers and function of T cells, supporting a primary defect in immunity rather than in barrier function.

This study has demonstrated an immunological abnormality that distinguishes BPF from all other forms of pemphigus. Thymosin alpha 1 levels may be important for epidemiological studies as it has been postulated that BPF is caused by an infectious agent. Further studies should enhance our understanding of the relationship between infection and autoimmunity.

## ACKNOWLEDGMENTS

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# An assessment of the diagnostic value of the monoclonal antibodies Leu 8, OKT9, OKT10 and Ki67 in cutaneous lymphocytic infiltrates

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## SUMMARY

Biopsies from 63 patients, 40 with mycosis fungoides (MF) and 23 with other lymphocytic infiltrates, were stained with a panel of monoclonal antibodies to differentiation antigens (OKT10, Leu 8), to activation antigens (OKT9), and to an antigen expressed by all dividing cells (Ki67) to establish whether this panel was of value in the diagnosis of mycosis fungoides. None of these antibodies used in isolation was found to give a staining pattern specific for mycosis fungoides, but the phenotype Leu 3a+, OKT9+, Leu 8− of the majority of lymphocytes in the cutaneous infiltrate was strongly suggestive. The presence of Leu 3a+ Leu 8− lymphocytes is of particular interest in that in the peripheral blood both of normal subjects and of MF patients Leu 3a+ lymphocytes also are predominantly Leu 8+, as were the lymphocytes in the cutaneous infiltrates of the non-MF infiltrates included in this study.

The availability of monoclonal antibodies raised against T lymphocyte subsets has stimulated several papers on the value of analysis of these cell types in cutaneous lymphocytic infiltrates, particularly in mycosis fungoides (MF). It is now generally agreed that the predominant cell type infiltrating the epidermis and dermis in the great majority of cases of MF bears the markers of the T helper subset (MacKie & Turbitt, 1982; Thomas *et al.*, 1982; Willemze *et al.*, 1983). However, this cell type is also that most commonly represented in the cutaneous infiltrate of both allergic and irritant contact dermatitis reactions (Scheynius *et al.*, 1983; Ferguson *et al.*, 1984). It would thus appear that quantitation of the T helper/T suppressor ratio in the MF infiltrate does not identify a pattern specific for MF. For this reason we have analysed the reactivity of a panel of newer monoclonal antibodies in sections from histologically proven MF, clinically suspected but not yet histologically definite MF, and non-MF lymphocytic infiltrates, to see if a more specific diagnostic pattern can be obtained.

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## METHODS

Sixty-six biopsies were used for the study (Table I). Fourteen were from patients with plaque stage proven MF, three from patients with histologically proven nodular or tumour stage MF, 18 from patients with clinically suspected but not yet histologically confirmed early MF, five from patients with plaque stage MF receiving PUVA treatment, and 23 from patients with other lymphocytic infiltrates. All biopsies were snap frozen, cut at 5 μm and used in a standard immunoperoxidase technique previously described (MacKie & Turbitt, 1982). The monoclonal antibodies used are detailed in Table 2. These included Leu 8, an antibody to a subset of regulatory T lymphocytes which also bear the surface antigen of either the T helper or the T suppressor cytotoxic subset (Gatenby *et al.*, 1982). This antibody has been used in analysis of lymphocyte subpopulations in the peripheral blood by Lanier *et al.* (1983). Ki67 is an antibody which recognizes an antigen expressed by all dividing cells in the G1, G2 and M phases of the cell cycle (Gerdes *et al.*, 1984). OKT9 recognizes the transferrin receptor which is a 90 000 dalton glycoprotein expressed on proliferating or activated cells, including foetal cells, adult bone marrow, and most malignant cell lines (Sutherland *et al.*, 1981). It is not seen on peripheral blood lymphocytes but is present on 10% of thymocytes (Kung *et al.*, 1981). OKT10 stains early thymocytes (Kung *et al.*, 1980; Reinherz *et al.*, 1980) and is lost in subsequent post-thymic maturation.

TABLE 1. Patients included in the study

| Diagnosis | No. of patients |
|---|---|
| Mycosis fungoides: | |
| Histologically definitive untreated plaque stage; | 14 |
| Histologically definite MF tumour stage; | 3 |
| Clinically suspected, histologically unproven untreated; | 18 |
| Definitive treated with PUVA | 5 |
| Non-Hodgkins non-MF lymphoma | 3 |
| Jessner's lymphocytic infiltrate | 5 |
| Chronic superficial dermatitis | 2 |
| Dermatitis | 4 |
| Drug eruptions | 2 |
| LE (Discoid) | 2 |
| Lymphomatoid papulosis | 5 |

In every case, staining with a standard battery of monoclonal antibodies against pan T cell, T helper, T suppressor and Langerhans cells was carried out, and a double staining immunoperoxidase method (MacKie & Turbitt, 1982) was used in some cases to further classify the lymphocytes in the infiltrate.

The antibodies Leu 7, which recognizes NK cells, and OKM1, which recognizes monocytes, were used either in the double staining technique or on sequential sections when either Leu 8+

TABLE 2. Monoclonal antibodies used in the study

| Antibody and source | Cells stained | Working dilution |
|---|---|---|
| OKT 9 (Ortho) | Transferrin receptor. Activated T & B cells. Monocytes, early thymocytes. | 1:50 |
| OKT10 (Ortho) | T thymocytes and bone marrow precursor cells including B cells, monocyte and myeloid precursors. | 1:100 |
| Leu 8 (Becton-Dickinson) | Defines a regulatory subpopulation of T cells on both helper and suppressor subsets. Also reacts with monocytes and granulocytic cells. | 1:100 |
| Ki67 (J Gerdes, Kiel University) | Antibody to a proliferation antigen found in the nuclei of cells in active cell division (S, $G_2$ and M phases). | 1:20 |
| OKM1 (Ortho) | Monocytes. | 1:100 |
| Leu 7 (Becton-Dickinson) | NK cells (large granular lymphocytes). | 1:100 |
| Leu 4 (Becton-Dickinson) | Pan T lymphocyte marker | 1:100 |
| Leu 3a (Becton-Dickinson) | T helper lymphocyte marker | 1:100 |
| Leu 2a (Becton-Dickinson) | T suppressor cytotoxic marker | 1:100 |
| NA 1 34 (Prof. A. McMichael) | Langerhans cell marker | 1:100 |

OKM1 and Leu 7 were used as additional markers to identify and eliminate monocytes and NK cells from OKT10 and Leu 8 positive counts.

or OKT10+ cells were seen. This is necessary because NK cells react weakly with both Leu 8 and OKT10, and OKT10 also stains cells of the monocyte-macrophage series. Cells stained with either Leu7 or OKM1 were therefore subtracted from the Leu8+ and OKT10+ populations to give a true value for the lymphocyte population stained by these antibodies.

Because of our initial observations with the monoclonal antibody BE2, originally produced against malignant T cells, but in our hands reacting also with capillary endothelium, we elected not to carry out a detailed analysis with this antibody.

## RESULTS

The results are shown in Table 3. No single antibody gave a staining pattern which differentiated biopsies considered on routine pathological examination to be either diagnostic or suggestive of MF from other lymphocytic infiltrates. The majority of biopsies in all conditions studied showed some positive staining of lymphocytes with the transferrin receptor antibody (OKT9). The proportion of lymphocytes stained varied from 5%–80% and, although the figure tended to be higher in biopsies with a definitive MF pattern (Fig. 1), OKT9 staining was not a useful discriminator taken in isolation.

TABLE 3. Results of immunoperoxidase staining with monoclonal antibodies

| Diagnosis | % of infiltrates stained—mean (range) | | | | | | |
|---|---|---|---|---|---|---|---|
| (No. of cases) | OKT9 | | OKT10 | | Leu 8 | | Ki67* |
| MF (14) | 43 | (10–80) | 1·4 | (0–5) | 28 | (1–60) | 2/12 |
| MF tumour (3) | 70 | (50–90) | 2 | (1–3) | 23 | (5–50) | 1/2 |
| MF on PUVA (5) | 9 | (0–15) | 11 | | 5·5 | (0–10) | 2/4 |
| Pre-MF and possible MF (18) | 14 | (1–25) | 1·4 | (0–5) | 14 | (1–60) | 2/12 |
| Non-MF lymphoma (3) | 30 | (5–75) | 0 | | 15 | (10–20) | 0 |
| CSD (2) | 10 | | 0 | | 10 | | 0 |
| Dermatitis (4) | 46 | (20–60) | 1 | | 0 | | 0 |
| Lymphomatoid papulosis (5) | 46 | (20–60) | 0·25 | (0–1) | 38 | (10–60) | 2/4 |
| Jessner's (5) | 10 | | 0 | | 70 | (50–80) | 1/4 |
| Drug eruptions (2) | 50 | (10–90) | 5 | | 1 | | 0 |
| LE (2) | 60 | (30–90) | 2 | | 10·5 | (10–15) | 0 |

* This column gives the number of cases in which a very small number of positive lymphocytes was seen (< 0·1%).

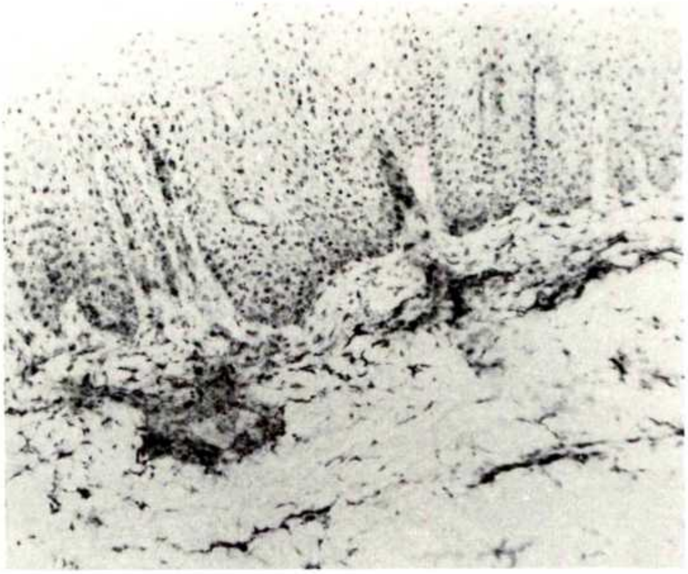

FIGURE 1. Diffuse OKT9 (transferrin receptor) staining in the papillary dermis around a cluster of lymphocytes. This pattern is seen commonly with OKT9 antibody and is *not* specific for either benign or malignant lymphoid infiltrates.

A very much smaller proportion of infiltrating lymphocytes stained with OKT10 in the majority of biopsies. The proportion was usually around 1% of all lymphocytes, but in a few biopsies from tumour stage MF nodules it was as high as 10%. Staining of sequential sections with OKM1 identified these OKT10 positive cells which were of the monocytic rather than the

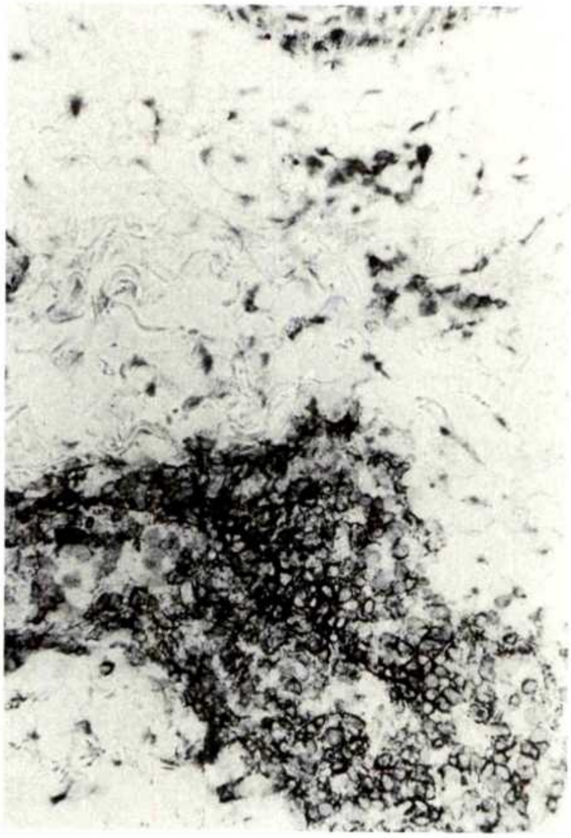

FIGURE 2. Leu 8-positive lymphocytes lying in a cluster in the papillary dermis in Jessner's lymphocytic infiltrate. 75% of the lymphocytes are positive, a proportion similar to that seen in the peripheral blood. In contrast, in mycosis fungoides less than 30% of the predominantly T helper lymphocytes are stained.

lymphocytic cell series. These were then subtracted to give the true OKT10-positive lymphocyte count. Leu 8-positive lymphocytes were seen in both large numbers and proportions in patients with Jessner's lymphocytic infiltrate (Fig. 2), and also in lymphomatoid papulosis. Double staining with Leu 8 and either Leu 2a (suppressor/cytotoxic cells) or Leu 3a (helper/inducer T cells) carried out in these cases showed that in the lymphomatoid papulosis cases, the Leu 8+ cells were also Leu 2a+ (suppressor/cytotoxic). This was not seen in Jessner's lymphocytic infiltrate where the Leu 8+ lymphocytes were also Leu 3a+.

Positive staining with Ki67, which recognizes an antigen expressed on dividing cells, was observed virtually always in a proportion of keratinocyte nuclei in the basal layer of the epidermis. Small numbers of Ki67-positive lymphocytes were seen scattered in the dermal infiltrate in relatively few cases of MF (2/12) (Fig. 3) and pre-MF (2/2), and also in two of the four cases of lymphomatoid papulosis. No Ki67+ cells were seen in the other lymphocytic infiltrates.

Leu 7, an antibody to NK cells (large granular lymphocytes), stained faintly only a very few cells (1%–4%) in the 16 cases which required to be examined with this antibody, indicating that the majority of Leu 8+ and OKT10+ cells were not NK cells. Similarly, very few isolated OKT10+ cells stained with OKM1.

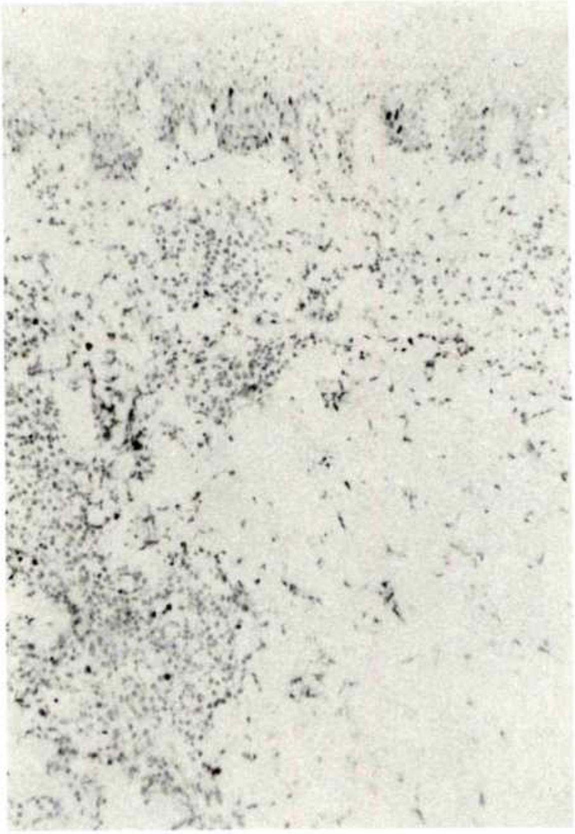

FIGURE 3. Ki67 preparation. The densely stained nuclei in the basal layer and in a few cells in the lymphocytic infiltrate indicate cells in $G_1$, $G_2$ or M phase of the cell cycle. Biopsy from a patient with advanced nodular mycosis fungoides.

PUVA treatment generally results in a marked decrease in cell numbers in the dermal infiltrate. Thus, the low numbers of OKT9+ and Leu 8+ cells in the five cases of MF on PUVA treatment studied, are assumed to be due to loss of the cells bearing these markers.

## DISCUSSION

The analysis of cutaneous infiltrates using antibodies to the major T lymphocyte subsets of helper and suppressor cytotoxic cells has so far failed to give a pattern which will distinguish MF or pre-MF from other conditions characterized by a dermal lymphocytic infiltrate. This has led to a study of the possible value of other cell markers not restricted to T cells which might both be more specific for MF and also reveal evidence either of activation or of a differentiation change in the MF T cell infiltrates.

The transferrin receptor recognized by OKT9 is present on 10%–80% of lymphocytes in the MF biopsies in this series and has been demonstrated also in cases of MF studied by other workers (Kung *et al.*, 1981; Haynes, Hendry & Jegasothy, 1982; MacMillan *et al.*, 1982). We have also demonstrated this marker on 20%–60% of lymphocytes in dermatitis reactions. The presence of OKT9 staining alone is not therefore a marker with specificity for an MF infiltrate.

OKT10-positive lymphocytes have been reported in T cell acute lymphocytic leukaemia (Reinherz *et al.*, 1980). The antigen recognized by OKT10 also occurs on the monocyte series and on natural killer (NK) cells. In this study, the numbers of monocytes and NK cells were determined by both double and sequential staining with OKM1 and Leu 7 respectively and the OKT10-positive lymphocyte count was then adjusted by subtraction. Our low incidence of positivity at 1·4% for MF is slightly less than the figure of 3% reported by Chu *et al.* (1984). This indicates that as far as expression of this surface antigen is concerned, the majority of T lymphocytes in MF do not represent 'dedifferentiated' cells or lymphocytes which have been blocked at the thymocyte stage of differentiation.

The monoclonal antibody Ki67 provides an opportunity to identify dividing cells (Gerdes *et al.*, 1984). Apart from the dividing keratinocytes in the basal layers of the epidermis, a proportion of which were positive in all sections studied, there were surprisingly few positive cells in the infiltrates even in the nodules of tumour stage MF. This would tend to support the hypothesis that the cutaneous lesions in MF are the result of an accumulation of infiltrating cells which have divided elsewhere rather than a rapidly dividing intradermal T lymphocyte population.

Within both the helper and suppressor T lymphocyte populations there is a subset of regulatory T cells which bear a surface antigen recognized by Leu 8 (Gatenby *et al.*, 1982). In populations of normal T cells from peripheral blood approximately 50%–80% of Leu 2a+ (suppressor/cytotoxic cells) and 75%–90% of Leu 3a+ (helper/inducer T cells) are also Leu 8-positive (Lanier *et al.*, 1983). In peripheral blood from an MF patient, we found 77% of all circulating T lymphocytes to be Leu 8+ on FACS analysis, proportions similar to those found in peripheral blood from healthy individuals. A high proportion of Leu 8+ lymphocytes would therefore be expected in the dermal infiltrate of MF if the cells in the dermis and epidermis were simply an 'overspill' from the peripheral blood cells. The fact that the majority of lymphocytes in the MF infiltrate were Leu 3a+, Leu 8− indicates that this is not the case. From *in vitro* studies (Gatenby *et al.*, 1982; Ballieux & Heijnen, 1983; Lanier, *et al.*, 1983) using autologous MLR as a T cell-dependent stimulus for immunoglobulin synthesis, it appears that the major helper effect lies within the numerically minor Leu 3a+8− subset, and also that neither Leu 2a+8+ nor Leu 2a+8− cells alone suppress immunoglobulin synthesis, but in combination these subsets are markedly inhibitory. The Leu 3a+8+ subset of cells induce or 'help' unprimed T cells to differentiate into T suppressor effector cells.

Functional studies are needed to establish the significance of a T helper lymphocyte population which is predominantly Leu 8− in the skin of MF patients. Our observations with the Leu 8 antibody in the skin and blood of these patients would suggest, however, that there is specific and preferential infiltration of the skin by a subset of T helper lymphocytes in a manner which does not suggest a simple overspill from the peripheral blood. We therefore conclude that the addition of the antibody Leu 8 to the standard lymphocyte profile may be of value in the assessment of possible MF.*

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* While this paper was in preparation for publication, a further study of the expression of Leu 8 in MF was published by Abel *et al.* (1985), with similar findings.

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# Leukapheresis in the treatment of cutaneous T-cell lymphomas

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## SUMMARY

In six patients with cutaneous T-cell lymphomas (CTCL), the effect of leukapheresis (LPH) on the disease and on various immunological and haematological parameters was investigated. Enriched mononuclear cells were removed by continuous centrifugation leukapheresis from the peripheral blood of the patients. A profound and longlasting effect was seen in one patient with the Sézary variant; a moderate response was seen in two other Sézary patients. No benefit was observed in two patients with more aggressive leukaemic disease, and one patient with plaque stage MF responded on two occasions. No limiting side effects were recognized and no consistent changes of immunological or haematological parameters were observed after LPH.

Therapy of cutaneous T-cell lymphomas (CTCL), a term introduced by Edelson (1975) to designate a group of diseases including Sézary syndrome, mycosis fungoides and related T-cell neoplasms, is very unsatisfactory. CTCLs are characterized by a proliferation of lymphocytes in the skin. Several investigators have used leukapheresis (LPH) to treat patients with CTCL, based on the assumption that removal of large quantities of lymphocytes, including Sézary cells, from the peripheral blood would reduce the number of abnormal T cells in the skin. We report a patient with Sézary syndrome treated for more than 66 months with LPH during which time he achieved partial remission without further progression of the disease. Encouraged by this result, we used LPH as monotherapy in five additional patients with different types of CTCL. The clinical course, the effects on peripheral blood cell counts and various immune parameters are described.

## METHODS

A continuous flow centrifugation leukapheresis apparatus (LPH, IBM 2997 Cell Separator) was used to remove leukocytes from the peripheral blood. Details of the method, the yield and purity of the cells obtained have been described (Sorg & Knop, 1983). The average yield was approximately $3{\cdot}3 \times 10^{10}$ leukocytes per LPH, containing 70% mononuclear cells. The mononuclear cells contained 10–55% monocytes and 45–90% lymphocytes. An analysis of the

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collected mononuclear cells with monoclonal antibodies of the OKT series demonstrated an enrichment of OKT4+ cells in the leukocyte concentrate as compared to the unseparated blood. Complete blood cell counts were performed before and after each LPH. Blood smears were stained with Pappenheim stain to detect and quantitate circulating atypical mononuclear cells; 400 cells were counted to achieve a given percentage. Sézary cells were also identified by semithin section microscopy. Routine examinations, liver and renal function tests, protein electrophoresis, quantitation of complement components and immunoglobulins were performed before and after LPH. In four patients, lymphocyte stimulation tests and immunoglobulin production *in vitro* were assessed.

### *Determination of T-cell subpopulations*

T4+ and T8+ cells were determined using monoclonal antibodies of the OKT series (Ortho Diagnostic System Inc., Raritan, NJ 08869, U.S.A.). The cells were quantitated by a fluorescence activated cell sorter (EPICS V, Coulter Electronics).

### *Lymphocyte stimulation assays*

Leukocytes were obtained by Ficoll-Hypaque separation of peripheral blood using standard methods. The cells were cultivated in MEM Dulbecco plus 10% human serum at 37°C and 7% $CO_2$. The cells were stimulated with concanavalin A (Con A) and phytohaemagglutinin (PHA), for 72 h. $^3$H-thymidine was added for the last 16 h and the incorporation of the radiolabel measured (Table 4).

### *Immunoglobulin production* in vitro

Peripheral blood leukocytes separated by Ficoll-Hypaque were stimulated with pokeweed mitogen (PWM) (Difco) for 7 days. The supernatants were tested for immunoglobulin A, G and M, using an ELISA test according to the method of Rissing, Buxton and Talledo (1980) (Table 4).

## PATIENTS

All relevant data concerning disease activity and stage of the disease of the six patients before treatment are summarized in Table 1.

### *Patient 1*

This male patient presented in February 1979, aged 66 years, with a 3-year history of pruritic exfoliative melanoerythroderma. Nontender lymphadenopathy had developed in the groin (approximate size 2·5 × 2·0 cm), followed by sternocleidomastoid and supraclavicular swellings of the lymph nodes (up to 1·5 × 2·0 cm). Sézary cells were detected on histological examination of lymph nodes. In the peripheral blood 13% Sézary cells were present and semi-thin sections of the buffy coat showed cells with convoluted and cerebriform nuclei. In addition, a monoclonal IgG lambda immunoglobulin was found. The diagnosis of Sézary syndrome was confirmed by histopathological and electron microscopic examination of the skin. This patient was treated initially with PUVA (a total of 680 joules/cm$^2$). He responded with a reduction of erythema and scaling, but after 18 months the disease relapsed and therefore therapy with LPH was started. The patient has been treated for over 66 months with LPH given at 4–5 week intervals. At present, he does not require any further therapy with the exception of occasional local application of a corticosteroid ointment. Erythema, infiltration and pruritus have improved

TABLE 1. Clinical data on six patients included in the study

| Patient | Sex | Age at onset (years) | Disease* duration | Clinical symptoms | Splenomegaly | Lymph node biopsy findings | Bone marrow examination | Peripheral WBC cells/mm$^3$ | Blood Sézary cells/mm$^3$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 62 | 4 years | erythroderma<br>lymphadenopathy<br>palmoplantar keratoderma<br>nail dystrophy | − | Sézary cells | nondiagnostic | 7 000 | 910 |
| 2 | M | 75 | 1 year | erythroderma<br>lymphadenopathy<br>nail dystrophy | + | Sézary cells | not done | 23 800 | 8 330 |
| 3 | M | 66 | 4 years | erythroderma<br>lymphadenopathy<br>palmoplantar keratoderma<br>nail dystrophy | + | Sézary cells | nondiagnostic | 32 000 | 6400 |
| 4 | M | 78 | 10 months | erythroderma<br>lymphadenopathy | − | dermatopathic lymphadenitis | not done | 10 500 | 1100 |
| 5 | M | 56 | 8 years | erythroderma<br>lymphadenopathy<br>palmoplantar keratoderma<br>nail dystrophy | + | Sézary cells | Sézary cells | 13 000 | 1040 |
| 6 | M | 45 | 2 years | generalized plaques covering about 54% of the skin surface<br>lymphadenopathy | − | dermatopathic lymphadenitis | not done | 5 000 | none |

* Approximate interval between first symptoms and first leukapheresis.

considerably, and scaling is absent. The previously enlarged lymph nodes are no longer palpable. Histology performed 5 years after starting LPH therapy showed a mild non-specific mononuclear cell sub-epidermal infiltrate. The patient still has 1–3 day periods of more intense pruritus and erythema every 4 weeks. Adverse effects of LPH therapy have not been seen other than an occasional slight drop of haemoglobin and erythrocyte count (Table 3).

*Patient 2*

This 75-year-old male patient presented with erythroderma, generalized pruritus, enlarged inguinal axillary and supraclavicular lymph nodes (up to 6·0 × 4·5 cm) and splenomegaly. The diagnosis of Sézary syndrome was confirmed by histology, which showed a dense, band-like infiltate of lymphocytoid cells in the upper dermis. Some eosinophils and plasma cells were admixed. The lymphocytes possessed hyperchromatic, irregularly shaped, markedly indented nuclei. There was epidermotropism with typical Pautrier microabscesses. The total white blood cell count was 23 800/mm$^3$ with 8330 (35%) Sézary cells/mm$^3$. He received a total of 9 LPH in 6 weeks. Despite LPH, the white blood cell count increased and the skin condition remained unchanged. LPH was stopped and cytostat treatment introduced. The patient died of septicaemia 5 months later.

*Patient 3*

This male 66-year-old patient had a pruritic, diffuse, scaly erythema on the trunk, plaques on the extremities and on the head and neck, and palmar and plantar hyperkeratosis. Inguinal and axillary lymph nodes were enlarged up to 2·0 × 1·5 cm. Histology confirmed the diagnosis of Sézary syndrome. Total white blood cell count was 32 000/mm$^3$ with 20% (6400 c/mm$^3$) Sézary cells. LPH treatment was started and although significant improvement of the skin condition was observed, the leukocyte count and the number of Sézary cells increased. LPH was stopped after 5 treatments. The patient died a few months later of a heart attack.

*Patient 4*

This male 78-year-old patient showed erythroderma, thinning of axillary and pubic hair and

TABLE 2. Effects of LPH on the clinical course of the disease

| Patient | Total no. of LPH | Erythema and scaling | Infiltration | Lymph-adenopathy | Pruritus | Course |
|---|---|---|---|---|---|---|
| 1 | 22 | + + | + + | + + | + + | still under treatment |
| 2 | 9 | – | – | – | – | died 5 months later from septicaemia |
| 3 | 5 | + + | + + | – | + | died 1 month later from unrelated cause (heart attack) |
| 4 | 8 | + | – | + | + | died 4 months later from unrelated cause (accident) |
| 5 | 19 | + | – | – | + | change of treatment |
| 6 (a): | 10 | + + | + + | + | + + | (a): relapse after 7 months |
| (b): | 8 | + + | + + | + | + + | (b): without skin manifestations for 8 months |

+ + Significant improvement.
+ Slight improvement.
– No change.

TABLE 3. Peripheral blood cell count on four patients before and after LPH

| | Patient number | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | | 3 | | 5 | | 6* | |
| | pre† | post† | pre | post | pre | post | pre | post |
| WBC (cells/mm$^3$) | 7000 | 7000 | 32 000 | 46 000 | 13 000 | 6400 | 5920 | 5000 |
| Lymphocytes (cells/mm$^3$) | 1470 | 1120 | 18 560 | 21 714 | 4680 | 3008 | 1416 | 750 |
| Sézary cells (cells/mm$^3$) | 910 | 560 | 6400 | 18 480 | 1040 | 896 | none | none |
| T4 positive cells (%) | n.d. | 40 | 67 | 62 | 72 | 77 | 32 | 20 |
| T8 positive cells (%) | n.d. | 8 | 2·0 | 0·5 | 10 | 3 | 18 | 9 |

* Values are related to second LPH-series (b series).
† Before LPH and after completing LPH treatment.

axillary and inguinal lymphadenopathy (3·2 × 2·0 cm and 4·0 × 2·5 cm, respectively). The white blood cell count was not significantly elevated, but 11% (1100/mm$^3$) Sézary cells were present. Histology confirmed the diagnosis of CTCL. Numbers of OKT8 + cells were increased in the peripheral blood and in the skin infiltrate. Eight LPH were performed over 3 weeks. Erythema and pruritus improved slightly but the patient died 4 months later of an accident unrelated to the disease.

### *Patient 5*

This male 56-year-old patient had generalized pruritus, erythroderma, palmar and plantar hyperkeratoses, axillary and inguinal lymphadenopathy (2·0 × 1·5 cm and 3·0 × 2·3, respectively) and splenomegaly. On admission, the white blood cell count was 13 000/mm$^3$ with 8% (1040 c/mm$^3$) Sézary cells. Histology confirmed the diagnosis of CTCL. He was treated with 19 LPH over a 4-month period. Although a slight improvement of erythroderma and puritus was observed, the skin condition remained troublesome.

### *Patient 6*

This 45-year-old male patient presented with pruritic large scaly plaques on the right breast, lower extremities, buttocks, forearms and hands, involving 54% of his skin surface. Inguinal lymph node enlargement (2·0 × 1·5 cm) was also present. The clinical diagnosis of mycosis fungoides was confirmed by histology and immunohistology. A series of 10 LPH was performed over a 3-week period. During this treatment dramatic improvement of the skin condition was observed, with complete clearing of most of the skin infiltrates. The skin remained in remission for about 7 months, then relapsed. A second series of eight LPH (2–3 per week) was given and some plaques disappeared, leaving hyperpigmentation, while others showed significant flattening.

### *Side-effects*

No side-effects were observed except for a transient hypocalcaemia.

### *Laboratory parameters*

LPH did not reduce the circulating cell numbers in Patients 2 (values not shown) and 3 with initially high white blood cell counts (Table 3). In Patients 4 and 5 a reduction of the total white blood cell count was observed. In Patient 4 (values not shown) a drop in Sézary cells from 11%–2% (1100–172/mm$^3$) occurred, whereas in Patient 5 Sézary cells decreased from 14%–8% (1040–896/mm$^3$). No significant changes in the helper and suppressor lymphocyte subpopulations were found after LPH. The effect of LPH on lymphocyte proliferation as measured by mitogen stimulation was variable (Table 4). In Patient 3, who initially showed a normal response, a significant decrease of lymphocyte proliferation was observed. In Patients 4 and 5, who had low values initially, an increase in lymphocyte proliferation was observed after LPH. In Patient 6, who had low normal values before LPH, no change was observed after LPH. LPH had no effect on immunoglobulin production *in vitro* (Table 4) or on serum immunoglobulin and complement levels (results not shown).

## DISCUSSION

The therapeutic effect of LPH in our patients was variable. Patient 1, who has been treated for more than 66 months, is not completely free of symptoms, but these are tolerable. In two patients (4 and 5) with normal white blood cell counts and a total of approximately 1000 Sézary cells per mm$^3$ transient benefit from LPH was observed. In two patients with Sézary syndrome (2 and 3) and high white blood cell count (> 20 000/mm$^3$), while improvement of the skin lesions was noticed, in both patients an increase of total cell numbers and Sézary cells was observed,

TABLE 4. Effect of LPH on lymphocyte stimulation and immunoglobulin (Ig) production *in vitro* in four patients

| | | Patient number | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | | pre | post | pre | post | pre | post | pre | post |
| Lymphocyte stimulation (cpm × 10$^3$)* | PHA† | 46·9 | 7 9 | 8 4 | 37·9 | 8·3 | 33·1 | 41·5 | 44·9 |
| | Con A | 71·9 | 13·4 | 15·1 | 24·7 | 4·5 | 29·6 | 23 9 | 31·2 |
| Ig-production, (ng/ml) | IgA | 125 | 150 | 300 | 250 | 50 | 50 | 75 | 50 |
| | IgM | 50 | 75 | 100 | 100 | 125 | 250 | 25 | 25 |
| | IgG | 100 | 100 | 200 | 250 | 100 | 250 | 500 | 600 |

* Mean of triplicate cultures with PHA 8 μg/ml or Con A 16 μg/ml; range in normal controls was 45–58 × 10$^3$ cpm for PHA and 35–48 × 10$^3$ cpm for Con A.

† Mitogen concentrations in the range 2–64 μg/ml for PHA and 4–128 μg/ml for Con A.

indicating a further progression of the disease under LPH. LPH may improve the skin lesions by reducing the cellular infiltrate. This is demonstrated clinically and histologically in Patient 6. This suggests that a success of LPH therapy may depend on the stage of the disease.

Our results are consistent with data in the literature. Edelson *et al.* (1974) reported an almost complete remission in one patient after LPH, but Safai, Reich and Good (1978) failed to produce any benefit with LPH in a patient with Sézary syndrome. Pineda and Winkelmann (1981) treated five patients with LPH, two of whom showed a major response when prednisone and low dose chlorambucil was added to the regime. Marcus and Adams (1983) found symptomatic relief and marked clearing of skin lesions in one patient with Sézary syndrome treated with LPH.

Intense LPH or LPH over a period of 5 years had only little effect on various laboratory parameters tested. Although there was a slight drop in the total white blood cell count and Sézary cells in some cases, the effects on mitogen-induced immunoglobulin production and T-lymphocyte subpopulations were minimal or absent. The effect of LPH on mitogen-induced lymphocyte proliferation was probably dependent on the type and stage of the disease. In mycosis fungoides (Patient 6) where low normal values were observed before LPH, no change occurred after LPH. In two Sézary syndrome patients (4 and 5) with low WBC and Sézary cell counts, LPH improved the low proliferative activity of the lymphocytes but this was not a stable effect as 3 months later the counts were as low as observed initially (results not shown). The decrease in activity from normal to abnormally low values in Patient 3 (Sézary syndrome) with high WBC and Sézary cell count during LPH may be explained by a further progression of the disease unaltered by this treatment.

Possible mechanisms of the benefit of LPH on CTCL include an establishment of an equilibrium between the blood pools of lymphocytes and the skin, and an immune modulatory effect of LPH on the abnormal T-lyphocytes. The first mechanism would appear to be of minor importance since it could not explain the long-term response in Patient 1 who was treated with LPH once every 4–5 weeks and the regression of plaques observed in mycosis fungoides.

The second mechanism seems plausible to explain the variable effects of LPH on CTCL. LPH has been shown to improve disease activity in rheumatoid arthritis (Karsh *et al.*, 1981; Karsh *et al.*, 1979; Wahl *et al.*, 1983), a disease characterized by T-cell dysfunction. Comparing two groups of patients selected by a high or low T-lymphocyte proliferative response, Wahl *et al.* (1983) found that clinical improvement correlated with an enhancement of the proliferative response to antigens. From this the authors concluded that clinical improvement is related to a modulation of immune functions.

The abnormal T-lymphocytes in CTCL may still be accessible to immune regulatory control by normal T-lymphocytes, depending on the differentiation stage at which the cells are arrested and LPH may affect the migratory and functional behaviour of the CTCL-lymphocytes reducing the numbers and inflammatory activity of the abnormal T cells in the skin for a limited period of time. Improvement of the response to mitogens observed in two patients with an initially low lymphocyte proliferation may support such a mechanism.

Our studies suggest that in selected cases of early CTLC, LPH may result in symptomatic relief and that patients in an advanced stage of the disease will not benefit from LPH alone.

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# Patterns of dermatology referrals in a general hospital

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## SUMMARY

A survey of 500 patients hospitalized in non-dermatology wards and referred for dermatological opinion is reported. In approximately 50%, the skin lesions were related to the presenting illness; in 36·6% of these patients the skin condition contributed substantially to the diagnosis of the systemic disease. This study emphasizes the considerable interface between cutaneous and systemic disease and the need for close co-operation between medical and dermatology departments. The implications for postgraduate education are highlighted, since such patients are infrequently seen in a dermatology out-patient setting.

The cutaneous manifestations of systemic disease provide one of the most rewarding aspects of a dermatologist's work. While patients with such problems present occasionally to a dermatology out-patient clinic, or, infrequently, to a dermatologist in private practice, they are most often referred from the medical and other wards of a general hospital. In some centres the dermatology department may be isolated geographically from the medical departments with limited co-operation between these sections.

The diagnostic value of skin manifestations may thus be overlooked in the investigation and management of seriously ill patients. The consequent delay in diagnosis, inflation of costs by inappropriate investigation, and patient discomfort will overshadow the missed opportunity for education of both physician and dermatologist. Furthermore, a permanent record and follow-up of these illuminating patients may evade data capture in routine out-patient and ward records. We set out, therefore, to evaluate our own experience, based on close collaboration with other departments in a general teaching hospital. The results of our analysis of 500 consecutive cases are presented and discussed in this paper.

## METHODS

Groote Schuur Hospital is one of two major teaching hospitals providing tertiary medical care for the Cape Province of South Africa and Namibia with occasional patients drawn from more

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distant areas such as Malawi or Mauritius. Tertiary care is defined as involving highly trained teams of physicians, nurses and other health professionals who provide care to the patient over a fairly extended period of time. It is also usually accompanied by the use of extensive technology. Groote Schuur Hospital, while complying with this definition, offers a full spectrum of care incorporating a general out-patient department and emergency services, as well as specialist clinics and wards. It is a general hospital with more than 1600 beds covering virtually every medical discipline. There is a relatively small number of paediatric beds since the majority of children attend an allied paediatric hospital and are seen in dermatology consultation there.

All of the routine in-patient dermatological referrals from other departments were seen initially by one of the authors (N.H.). Selected patients of unusual interest or with diagnostic problems were then seen by the Head of the Dermatology Department (N.S.) together with the entire full-time dermatology staff, including all the registrars. The patient's name, age, sex, hospital number, referring department and ward, date of referral, biopsy results, and initial and final diagnosis were recorded, together with an assessment of whether the cutaneous lesions contributed to the diagnosis of the underlying disease.

Five hundred consecutive cases seen between March 1983 and June 1984 were analysed with regard to all the above features and were allocated to one of 19 disease or diagnostic categories.

## RESULTS

The analysis of the data obtained is listed in Tables 1–5.

The sex ratio was 1:1. Sixty-three skin biopsies were performed, i.e. 12·6% of patients seen. The dermatological disorder was incidental to the patient's admission in 260 cases (52%) and related to the patient's admission in 240 cases (48%). In this latter group, in 88 cases (36·6%) the dermatological findings were considered to have contributed substantially to establishing the medical diagnosis. The total number of admissions to wards other than dermatology during the survey period was 86983. The overall referral rate was, therefore, 0·6% of total hospital admissions.

## DISCUSSION

Our findings highlight the diagnostic value of dermatological signs of systemic disease. The ready accessibility of skin lesions is not the least important aspect, yet these may be overlooked in the management of more dramatic life-threatening manifestations of disease.

The high proportion of referrals from internal medicine (45·6%) (Table 1) is to be expected because of the diverse and increasingly identified skin signs of systemic disease. The highest percentage of admissions referred came from haematology (6·7%). The large numbers of drugs these very ill patients received, their immunocompromised state and the cases of graft-versus-host disease all contributed to this preponderance. A disproportionate number of patients referred from the urology wards (Table 1) were elderly males with solar keratoses, basal cell carcinoma and squamous cell carcinoma which the urologists had become skilled at recognizing.

Young and Miller (1968) reported symptomatic dermatological disorders in 47% of non-geriatric patients compared with 31·2% of the general U.S.A. population found to be affected in a population survey (cited by Johnson & Stern, 1979). If similar percentages can be extrapolated to the South African population, it may be concluded that many in-patients with skin problems were not referred. This could be the result of the attendant doctor treating minor skin problems

TABLE 1. Analysis of referrals according to source

| Source (discipline) | Number | % of all dermatology referrals | Total admissions during survey period | % of admissions |
|---|---|---|---|---|
| Internal Medicine (including Renal, Cardiology, G.I.T., Endocrine & Respiratory) | 228 | 45·6 | 15892 | 1·4 |
| Haematology | 29 | 5·8 | 436 | 6 7 |
| Rheumatology | 19 | 3·8 | NA | NA |
| Neurology | 17 | 3 4 | 763* | 2·2* |
| General surgery | 53 | 10 6 | 12552 | 0 4 |
| Cardiothoracic surgery | 17 | 3·4 | 3121 | 0·5 |
| Urology | 17 | 3·4 | 2224 | 0 8 |
| Neurosurgery | 16 | 3·2 | NA | NA |
| Orthopaedics | 12 | 2·4 | 3470* | 0·3* |
| Ophthalmology | 6 | 1·2 | 3177 | 0·2 |
| E.N.T. | 5 | 1·0 | 4776 | 0 1 |
| Obstetrics & Gynaecology | 42 | 8·4 | 23042 | 0·2 |
| Paediatrics (including neonates) in nursery and paediatric surgery) | 22 | 4·4 | 4394 | 0·5 |
| Radiotherapy | 14 | 2 8 | NA | NA |
| Psychiatry | 3 | 0·6 | 107 | 2·8 |
| Total | 500 | 100% | 86983 | |

* Estimate based on 1984 admissions.
NA, figures not available.

or delaying attention to these in the greater concern for the severe illness necessitating the patient's admission to hospital.

It is interesting to compare the numbers and types of patients referred to us with those described in a letter by Sherertz (1984), who saw 705 in-patients in a year, of whom approximately 57% had common dermatoses usually seen in primary care settings. She attributed this high referral rate of banal skin disorders to the previous absence of a dermatology service. Cutaneous manifestations of systemic disease accounted for nearly one quarter of our referrals (Table 2), as compared with 9·4% of Sherertz's cases. The detection of seven malignant tumours (Table 2), including one malignant melanoma, emphasizes the importance of the examining physician maintaining a high suspicion of, and careful search for, skin tumours.

The skin signs of systemic disease were grouped into 11 diagnostic categories (Tables 3a–k). The infections include tropical diseases as well as six patients with tuberculosis (Table 3a). These comprise one patient with tuberculous pharyngitis, two with papulo-necrotic tuberculide, one tuberculous ulcer, one lymphadenitis and one patient with lupus vulgaris with tuberculous peritonitis. These cases reflect the high prevalence of tuberculosis in South Africa (Fourie, 1983). The existence of papulo-necrotic tuberculide is these days doubted in most

TABLE 2. Diagnoses of patients referred

| Diagnostic group | No. of diagnoses | % of 515 conditions diagnosed* |
|---|---|---|
| Skin signs of systemic disease | 121 | 23 5 |
| Dermatitis | 88 | 17·1 |
| Drug reactions | 54 | 10·5 |
| Superficial fungal infections | 36 | 7·0 |
| Miscellaneous | 35 | 6·8 |
| Psoriasis | 30 | 5·8 |
| Complication of treatment | 29 | 5·6 |
| Sun damage | 17 | 3·3 |
| Bacterial infections | 16 | 3·1 |
| Non-specific infections | 16 | 3 1 |
| Benign tumours | 13 | 2·5 |
| Warts | 12 | 2 3 |
| Herpes simplex | 9 | 1·7 |
| Urticaria (not clearly drug induced) | 8 | 1·5 |
| Malignant tumours | 7 | 1 4 |
| Leg ulcers | 7 | 1·4 |
| Parasitic infestation | 7 | 1·4 |
| Acne vulgaris | 6 | 1·2 |
| Herpes zoster | 4 | 0·8 |
| Total | 515* | 100·0 |

* Some patients had more than one diagnosis.

developed western countries, yet in South Africa we still see many patients with skin tuberculosis (Morrison & Fourie, 1974; Saxe, 1985), often in out-patient clinics.

The numbers of patients referred with SLE (Table 3e) does not reflect the true numbers of patients seen by the physicians, since the more common signs of malar flush and widespread discoid lesions are familiar to the rheumatologists and physicians who treat these patients and who tend to refer only the more problematic cases.

Cutaneous drug reactions accounted for 10·5% of our referrals, i.e. 0·06% of all admissions. If these figures are compared with the 2·5% reported by Arndt and Jick (1976) and 1·1% by Stewart, May and Cullen (1979), it is likely that not all the hospital drug reactions were referred. Furthermore, our patients were drawn from all departments, including obstetric and surgical patients, who tend to receive fewer drugs than the medical and paediatric in-patients of the above studies. Of interest was the absence of ampicillin reactions (Table 4). This could be explained either on the basis of antibiotic prescribing habits in the hospital or the notoriety of ampicillin in adverse reactions.

Knowledge and evaluation of the patterns of dermatological referral increases the diagnostic skills of both the dermatologist and the referring doctor. Of particular value is the clustering of cases; the referral of similar patients from diverse departments enables the identification of previously unrecognized cutaneous manifestations of systemic disease.

The usual figures given for dermatological problems in a primary care or general practice setting range from 20% to 25% of the patient population seen (Branch, Collins & Wintroub, 1983). In contrast, our referral rate of 0·6% of patients indicates that numerous selective factors

TABLE 3. Skin signs of systemic disease

| Disease categories | No. of diagnoses |
|---|---|
| (a) *Infections* | |
| Erysipelas | 7 |
| Tuberculosis | 6 |
| Tickbite fever | 2 |
| Secondary syphilis | 2 |
| Streptococcal erythema nodosum with cellulitis/septicaemia | 2 |
| Herpes zoster with meningism | 1 |
| Leprosy | 1 |
| Cutaneous amoebiasis | 1 |
| ? Coxsackie eruption | 1 |
| Staphylococcal scalded skin syndrome | 1 |
| Typhoid rose spots | 1 |
| Echthyma gangrenosum | 1 |
| Chlamydial eruption mimicking tickbite fever | 1 |
| Staphylococcal osteitis with urticaria | 1 |
| (b) *Hypersensitivity reactions* | |
| Henoch Schönlein purpura | 4 |
| Necrotizing vasculitis (idiopathic) | 3 |
| Erythema nodosum + rheumatic fever + TB | 1 |
| Serum sickness | 1 |
| Sweet's syndrome + rheumatoid arthritis | 1 |
| Erythema multiforme mimicking meningococcal septicaemia | 1 |
| Toxic epidermal necrolysis from chickenpox | 1 |
| Toxic epidermal necrolysis, cause unknown | 1 |
| Wegener's granulomatosis | 1 |
| Aortitis with cutaneous vasculitis | 1 |
| Infectious hepatitis—urticarial vasculitis | 1 |
| (c) *Skin signs of malignancy* | |
| Pachydermoperiostosis | 1 |
| Herpes zoster with adenocarcinoma of peritoneum | 1 |
| Papular urticaria + chronic lymphoblastic leukaemia | 1 |
| Pruritus + lymphoma | 1 |
| Carcinoid syndrome, leonine facies + flushing | 1 |
| Poikiloderma vasculare atrophicans | 1 |
| Bullous pyoderma in leukaemia | 1 |
| Skin nodules: chronic myeloid leukaemia | 1 |
| Pityriasis rotunda + breast cancer | 1 |
| (d) *Nutritional disorders* | |
| Malnutrition | 3 |
| Pellagra | 2 |
| Scurvy | 1 |

were operating in this study. With the exception of the letter by Sherertz (1984), we have been unable to find any similar study of in-patient dermatological referrals in the literature, although three excellent textbooks provide a valuable reference source for skin signs of systemic disease, (Braverman, 1981; Callen, 1981; Fitzpatrick *et al.*, 1979).

One of the main purposes of epidemiological studies of dermatological disease in an out-

TABLE 3 (*continued*)

| | |
|---|---|
| (e) *Systemic lupus erythematosus* | |
| Vasculitis | 3 |
| Leg ulcer | 1 |
| Oral ulcer + photosensitivity | 1 |
| Ulcerating papulosquamous/vascular lesions | 1 |
| Photosensitivity | 1 |
| Vitiliginous lesions | 1 |
| Abscess | 1 |
| Recurrent herpes simplex | 1 |
| Rheumatoid nodule + LE profundus | 1 |
| Vasculitis, oral ulcers, alopecia | 1 |
| Malar flush | 1 |
| Telangiectasia of breasts | 1 |
| Adult tinea capitis (not on immunosuppressives) | 1 |
| (d) *Other rheumatological disorders* | |
| Mixed connective tissue disease (MCTD) | 3 |
| MCTD + psoriasis | 1 |
| MCTD + vasculitic ulcers | 1 |
| Systemic scleroderma | 2 |
| Doughy pale skin in rheumatoid arthritis | 1 |
| Psoriasis and ankylosing spondylitis | 1 |
| ? Dermatomyositis | 1 |
| Still's disease | 1 |
| Fibrosing alveolitis + acrosclerosis | 1 |
| (g) *Cardiac disease* | |
| Osler's nodes | 1 |
| Acute staphylococcal endocarditis + septic emboli | 1 |
| Erythema marginatum | 1 |
| Congestive cardiac failure with purpura | 1 |
| Chlamydial S.B.E. with papular eruption | 1 |
| (h) *Endocrine disease* | |
| Diabetic scleredema | 1 |
| Myxoedema—dry scaly skin | 1 |
| Necrobiosis lipoidica diabeticorum | 1 |
| Alopecia; hypothyroidism + acromegaly | 1 |
| Alopecia; panhypopituitarism | 1 |
| Skin tags, hirsutism, tooth spacing: acromegaly | 1 |
| Pancreatic panniculitis | 1 |

patient setting has been to identify the commonest dermatological diseases encountered, with a view to appropriate and relevant teaching at undergraduate level (Branch, Collins & Wintroub, 1983; Findlay & Park, 1969; Johnson & Stern, 1979).

However, while out-patient surveys are valuable in undergraduate teaching and vocational training for general practice, inpatient referrals provide a great deal of information previously unevaluated. It would be inappropriate to teach cutaneous medicine in its entirety to undergraduate students or even to general practitioners, yet it is seldom specifically taught to physicians and other non-dermatologists.

A substantial number of the 36·6% of patients in this study, whose dermatological findings

TABLE 3. (*continued*)

| | |
|---|---|
| (i) *Metabolic disease* | |
| Pruritus—cholestatic | 2 |
| Pruritus—iron deficiency | 1 |
| Porphyria cutanea tarda (typical skin lesions) | 1 |
| Variegate porphyria (onycholysis) | 1 |
| Alopecia—iron deficiency | 1 |
| Old tuberous xanthoma (normolipaemic) | 1 |
| (j) *Genetic disease* | |
| Neurofibromatosis | 3 |
| Pseudoxanthoma elasticum | 2 |
| Gingival fibromatosis, hypertrichosis and breast hypertrophy | 1 |
| Familial cold urticaria | 1 |
| Epidermolysis bullosa | 1 |
| Tuberous sclerosis | 1 |
| (k) *Other conditions* | |
| Behçet's syndrome | 2 |
| Sarcoidosis | 2 |
| ? Immunodeficiency | 1 |
| Uraemic dry skin | 1 |
| Nephritis with cutaneous vasculitis | 1 |

established the medical diagnosis, had not had this diagnosis suspected by the referring doctor. The precise figure in which the dermatologist alone made the diagnosis is unavailable since the purpose of this study was not an attempt to evaluate the performance of our colleagues. Rather, the dermatological skills required by a physician today may be possible only with the co-operative consultation that occurs in a teaching hospital between the various specialists and dermatologists.

From personal experience of earlier years, it was noted that delay often resulted from inaccurate diagnosis and inappropriate investigations of an ill patient with diagnostic cutaneous lesions. A close working relationship of most departments, particularly the Department of Medicine with the Department of Dermatology, and appreciation of the diagnostic value of skin lesions, has largely eliminated this situation in our own institution.

Our study does not purport to be epidemiological, since any attempt to assess the true incidence or prevalence of disease must be related to a clearly defined population. Appraisal of the different diagnostic groups referred, however, reveals the diseases and diagnostic pitfalls likely to be encountered in a particular medical or surgical setting, enabling the dermatologist to plan more effective teaching at postgraduate level (Tables 1–5).

Cutaneous manifestations in the group of patients studied differ from those seen in either a dermatology out-patient clinic or in dermatological private practice in South Africa. Moreover, the experience gained may be very different from that of some academic dermatologists in western countries such as the United State of America, who may actually see so few patients that the loss of clinical skills is feared (Caplan, 1984).

Many of the clinical problems seen in this study were those of complicated multisystem disease, and the diagnosis often evolved over a period of time, emphasizing the need for close

TABLE 4. Drug reactions

| Drug | Number of reactions | Reaction |
|---|---|---|
| Corticosteroids | 6 | Acne |
| Amiloride/hydrochlorothiazide | 5 | Vasculitis 2; erythema multiforme 1; photosensitivity 1; not described 1 |
| Cotrimoxazole | 5 | Erythema multiforme 3; vasculitis 1; fixed drug eruption 1 |
| Phenytoin | 4 | Macular erythematous eruption |
| Phenobarbitone | 3 | Morbilliform 1; toxic epidermal necrolysis 1; erythema multiforme 1 |
| Cefotaxime | 3 | Not described (multi-drug situations) |
| Methotrexate | 3 | Ulceration 2; folliculitis 1 |
| Allopurinol | 2 | Stevens Johnson syndrome 1; vasculitis 1 |
| INH (isonicotinic acid hydrazide) | 2 | Acne |
| Iodinated contrast media | 2 | Acne |
| Cytosine arabinoside (high dose) | 1 | Inflammation of hands (acral erythema) |
| Gold | 1 | Oral ulcers |
| Phenolphthalein | 1 | Fixed drug eruption |
| Carbamazepine | 1 | Morbilliform eruption |
| Tobramycin | 1 | Not described |
| Penicillin | 1 | Urticaria |
| Pancreatic enzyme supplements | 1 | Vasculitis |
| Rauwolfia/Hydroflumethiazide | 1 | Photosensitivity |
| Propranolol | 1 | Psoriasis |
| Erythromycin | 1 | Urticaria |
| Flucloxacillin | 1 | Morbilliform eruption |
| Unidentified | 8 | |
| Total | 54 | |

TABLE 5. Complications of therapy

| | |
|---|---|
| Graft-versus-host disease | 9 |
| Contact dermatitis from local therapy/procedure | 6 |
| Opportunistic infection (warts, tinea, candida) | 3 |
| Impetiginized radiodermatitis (? benzocaine reaction) | 2 |
| Emboli from thrombosed renal shunt | 2 |
| Allergy to thiuram in haemodialysis machine | 1 |
| Thrombocytopenic purpura (leukaemic induction) | 1 |
| Erythema multiforme from radiotherapy | 1 |
| Folliculitis from shaving pre-op | 1 |
| Drip site ulcer | 1 |
| Keloid in laparotomy scar | 1 |
| Lymphoedema of abdomen after hysterectomy | 1 |
| Total | 29 |

co-operation and follow-up information. Not infrequently, a skin biopsy provided the requisite diagnostic information. The value of this very accessible and simple investigation has been underrated previously in disciplines outside of dermatology.

It is well known to dermatologists that skin biopsy increases the yield of diagnostic information proportionately to the accuracy of the clinical dermatological assessment. Therefore, it must be emphasized in the training of non-dermatologists that a skin biopsy does not replace a specialist clinical opinion, as the correlation of the clinical with the pathological information provides a more accurate dermatopathological diagnosis. A skin biopsy, performed in 12·6% of all our referrals, usually served to narrow the differential diagnosis or to confirm an informed clinical suspicion, rather than to provide a diagnosis which was totally unsuspected clinically. Other simple investigations, including skin scraping for microscopy and fungal culture, Tzanck smears and ophthalmoscopic investigation of nail fold capillaries (Minkin & Rabhan, 1982), were also valuable and easily performed and deserve to be taught to non-dermatologists.

The aspects of the teaching of dermatology that should be included in the postgraduate training of physicians are the management of common skin disorders such as eczema, psoriasis, acne, fungal infection and drug reactions. The diagnostic value of skin lesions in systemic disease, as well as the benefit of dermatological diagnostic procedures, should be stressed in the training of physicians.

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# Brachioradial pruritus: a tropical dermopathy

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## SUMMARY

Data on 42 patients with brachioradial pruritus seen in Hawaii over a 2-year period are presented. In contrast to previous reports, the sex ratio was 1:1 with a preponderance of patients in the younger age groups. All patients had developed the disorder after having lived in Hawaii for at least one year. More than half the cases had no history of neck trauma or arthritis, ruling out a peripheral neuropathy as the cause. Radiographs of 15 patients showed changes only in the patients in the older, arthritis-age groups. Brachioradial pruritus appears to be peculiar to the tropics.

Chronic intermittent pruritus localized to the flexor surface of the elbow where the brachial and radial nerves run has been infrequently described. The disorder, variously termed 'brachioradial pruritus' (BRP) (Heyl, 1983), 'solar pruritus of the elbows' or 'brachioradial summer pruritus' (Waisman, 1968), and 'prurit solaire' (Jean Mougin), has been attributed to sun exposure (Waisman, 1968), cervical spine disease, or local nerve damage (Heyl, 1983). All descriptions, other than isolated case reports, have originated from the subtropics, namely South Africa (Heyl, 1983) and Florida (Waisman, 1968). Since 1983, we have recognized BRP in 42 patients in Hawaii.

## METHODS

All patients were seen in a private dermatology practice on Kauai, the most westerly of the major Hawaiian islands. Since 1981, all diagnoses from this practice have been entered in a registry to facilitate case retrieval. Demographic data were recorded for all cases seen. These were supplemented by telephone interviews whenever possible for patients with BRP.

Kauai, situated in the tropics below latitude 23·5° North, has a mean temperature of 24·4°C (76° F) throughout the year. The permanent population of 44 800 comprises 28% Caucasian, 26% Philipino, 25% Japanese, 15% Hawaiian and part-Hawaiian individuals, and 6% from other groups (State Department of Planning, 1984).

## RESULTS

Brachioradial pruritus was diagnosed in 42 patients in 2 years, from March 1983 to February

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TABLE 1. Dermographic details of 42 patients with BRP

| | | No. of patients |
|---|---|---|
| Age (years) | < 19 | 1 |
| | 20–39 | 19 |
| | 40–59 | 17 |
| | 60–79 | 4 |
| | > 80 | 1 |
| Sex | Male | 20 |
| | Female | 22 |
| Race | Caucasian | 27 |
| | Japanese | 11 |
| | Philipino | 1 |
| | Hawaiian | 3 |

1985, inclusive. Complete data were compiled for 31 patients. For 11 patients only age, sex, race and date of diagnosis were available.

All patients experienced intense pruritus of one or both arms on the sun-exposed skin under which lie the brachial and radial nerves and the brachioradial muscle. The episodic symptoms were commonly described as a 'crawling under the skin' or a 'pins and needles itch' sensation. No diurnal pattern was described as has been reported in other studies (Waisman, 1968), but some patients complained that symptoms interfered with sleep. Pruritus preceded skin lesions in all cases. Forty-five percent had symptoms limited to the left arm, 13% to the right, and 42% suffered itching on both arms. Four patients had additional pruritic areas (shoulder, right anterior thigh and shin, scapula and buttock).

Onset of symptoms preceded presentation by one month to 15 years. The mean duration of episodic symptoms was 38 months and the median was 26 months. With one exception, all patients had lived in Hawaii for at least one year prior to developing BRP.

Demographic data for age, sex and race are presented in Table 1. The skin types of 37 patients

TABLE 2. Skin types of patients with BRP

| Skin type | | No. of patients |
|---|---|---|
| I. | Always burn, never tan | 1 |
| II. | Always burn, slight tan | 9 |
| III. | Sometimes burn, slight tan | 9 |
| IV. | Never burn always tan | 7 |
| V. | Heavily pigmented | 11 |
| VI. | Black | 0 |

are shown in Table 2. Five patients were taking hydrochlorothiazide, a potentially phototoxic medication.

Of 31 patients retrospectively interviewed, 39% noted exacerbation with sunlight, 48% identified no aggravating factors, and 13% blamed other factors (ingestion of meats, carrying a handbag on the affected arm, topical aloe vera, and pregnancy). Cutaneous findings varied from no objective changes to mild erythema, flesh-coloured papules, mild to moderate lichenification, and occasionally excoriations. Marked xerosis of the affected area was observed consistently in most patients.

Because Heyl (1983) impugned peripheral neuropathy from cervical spine disease as a putative cause of BRP, patients were questioned about history of antecedent neck trauma or disease. Fifty-eight percent had a negative history, 13% a history of cervical arthritis, and 19% a history of neck trauma; 10% were seeing a chiropractor or had a 'pinched nerve'. Cervical spine X-rays under an experimental protocol were offered to 31 patients. Fifteen patients consented to PA, lateral and oblique radiographs of the cervical spine. Five showed no abnormality, five showed disc narrowing, mainly of C5/6 and C6/7 discs, and the remainder who were all over 50 years old, showed assorted degenerative changes.

## DISCUSSION

Patients complaining of pruritus localized to specific anatomical areas occasionally present to dermatologists. When these symptoms are accompanied by lichenification and excoriation the practitioner may affix the label 'neurodermatitis' or lichen simplex chronicus and delve no deeper. Indeed, some patients to whom we and other Hawaiian dermatologists have given these diagnoses have since been recognized as suffering from BRP since Heyl (1983) resurrected this clinical entity.

BRP appears to be identified more commonly in tropical rather the temperate regions and occurs on sun-exposed sites. Therefore, it is reasonable to consider it a photodermatitis. The majority of BRP patients were Caucasian with skin Type II or III. However, 11 patients were Orientals who, by definition, have skin Type V (Fitzpatrick, 1979). Thus, although pigmentation may offer some protection from developing the disorder, it is far from absolute.

It is clear that BRP differs from other light-induced disorders in many important ways. It is significant that all but one sufferer were long-term residents of Hawaii. This suggests that chronic rather than acute exposure to solar radiation may be causative. Whether UVB, UVA, visible, or infra-red wavelengths precipitate the reaction is speculative. While five patients were taking hydrochlorothiazide, the signs and symptoms of BRP have no relationship to phototoxic drug reactions.

The physical findings are non-specific and no primary lesions, with the exception of a localized xerosis, have been identified. The xerosis may be a clue that subtle damage to cutaneous nerve endings has occurred, which may explain the symptoms leading to the secondary findings of lichenification and excoriation.

After prolonged exposure, UV radiation causes inflammatory and degenerative changes throughout various cutaneous layers, including the level where receptors are located (0·2 mm from the epidermal surface) (Fitzpatrick, 1979). Winkelmann and Weddell (quoted by Fitzpatrick, 1979) demonstrated hyperaesthesia and hyperexcitability to itch in areas of lichenified skin, with a decreased number of nerve fibres. Extended periods of intense solar irradiation may have produced these symptoms in our patients by similar mechanisms.

Heyl (1983) was the first to consider disease of the cervical spine as a cause of BRP. Most of his

14 patients were male, and in age groups subject to degenerative bone disease. In our study, the sexes were equally represented, and a substantial number were in the young age groups. If BRP were a neuropathy, sensory symptoms should be present over the entire dermatomal area and not be localized to a small area. Radiological evidence of cervical spine disease from C3 to T1 was abundant in this study, with no consistent pattern. These spinal nerves innervate a much wider area than that where skin pathology was found. Radiological findings of cervical spine disease are a common finding in all older patients. This explains why all our patients over the age of 50 had radiological evidence of pathology, while in those less than 50 years old, 60% were normal. We feel that cervical spine disease is not the cause of BRP but rather an epiphenomenon which reflects the population who manifest the disorder. This does not negate the possibility that BRP is a disease of the nervous system. It may be a photoneurological disorder; prolonged sun exposure in susceptible persons may cause damage to nerve endings which results in pruritus and altered sensation.

Notalgia paresthetica (hereditary localized pruritus) (Massey & Pleet, 1979), and meralgia paresthetica (Comings & Comings, 1965), are previously described, poorly understood, forms of localized pruritus, and BRP may well belong to this group.

BRP is common in residents of the tropics and subtropics. In view of this, and the fact that the disease manifests itself on sun-exposed areas, it is reasonable to postulate that BRP is at least in part a photosensitivity disorder. The mechanism(s) by which light may be a cause of BRP remains obscure.

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# Elastase, a marker for neutrophils in skin infiltrates

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## SUMMARY

The enzyme elastase (EC 3.4.21.37) has proved to be a convenient and extremely sensitive marker for the quantification of neutrophils in cutaneous infiltrates. Fluorometric assay using the synthetic substrate MeOSuc-Ala-Ala-Pro-Val-N-methylcoumarin permitted the measurement of this enzyme in as few as five cells and was linear up to about 1000 cells per sample. The mean activity of lysates of human blood-derived neutrophils was $0{\cdot}57 \pm 0{\cdot}08$ pmol of 7-amino-4-methyl-coumarin released per hour per neutrophil. Extracts of normal human skin contained no measurable elastase activity but resulted in a slight inhibition of the neutrophil enzyme (mean 12%).

Application to the *in vivo* situation has been demonstrated by the use of leukotriene $B_4$ as chemotactic agent. A reproducible neutrophil infiltrate was found at a dose of 2 ng, well below the threshold for the appearance of microabscesses.

Infiltration by neutrophils is a characteristic feature of many skin lesions. Since the density of the infiltrate provides an indication of the local intensity of the disease process, a method for the quantification of these cells would clearly be of value. It has already been shown that myeloperoxidase can be used as a specific marker enzyme for this purpose (Bradley *et al.*, 1982), but unfortunately the sensitivity of the technique described was not sufficient to permit its routine application to human biopsy material.

We have explored recently the use of an alternative enzyme, elastase (EC 3.4.21.37). Like myeloperoxidase, elastase is very specific for the polymorphonuclear leukocyte and is present in large quantities in the granules (Briggeman *et al.*, 1984; Gallin, 1984). It has the additional advantage, however, that a simple and exceedingly sensitive fluorometric assay is available (Castillo *et al.*, 1979). Here we present data to show that elastase is indeed a convenient and sensitive marker for blood neutrophils, and describe a pilot study to demonstrate that it can be used to quantify infiltrating neutrophils in small biopsies of human skin.

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## METHODS

### *Materials*

The substrate, MeOAuc-Ala-Ala-Pro-Val-N-methylcoumarin, was obtained from Bachem, Bubendorf, Switzerland. Leukotriene $B_4$ ($LTB_4$) was purchased from Paesel GmbH, Frankfurt, Germany. All other chemicals were of the highest grade available and were obtained from E.Merck, Darmstadt, Germany.

Blood was obtained by venepuncture of healthy volunteers, and polymorphonuclear leukocytes isolated by standard procedures (Trush, Wilson & van Dyke, 1978). The washed cells ($>90\%$ neutrophils) were lysed by sonication in a buffer containing 0·3% cetrimide, 0·1 M Tris and 1 M NaCl (pH 8·5) at a concentration of $10^6$ cells/ml. All dilutions were made in this same buffer.

### *Elastase assay*

Elastase was determined by the fluorescence of 7-amino-4-methyl-coumarin released from the substrate MeOSuc-Ala-Ala-Pro-Val-N-methyl-coumarin (Castillo *et al.*, 1979). A 10 mM stock solution of the substrate in dimethylsulphoxide was diluted to 250 $\mu$M with cetrimide buffer (above) on the day of use. The reaction was initiated by the addition of 20 $\mu$l of the diluted substrate to 20 $\mu$l aliquots of cell or skin extracts in 4 ml plastic tubes. A blank (20 $\mu$l substrate plus 20 $\mu$l cetrimide buffer) was always included. After incubating the covered tubes at 37°C for 1 h, the reaction was stopped by cooling in ice and diluting to 1 ml with carbonate-bicarbonate buffer (0·1 M, pH 10·5). Fluorescence was measured at exitation 375 nm, emission 440 nm using a Perkin-Elmer LS 5 spectrofluorometer. Enzyme activity was calculated by reference to a standard solution of 7-amino-4-methylcoumarin in carbonate-bicarbonate buffer (100 pmol/ml).

Preliminary experiments verified that the reaction rate was linear with time for at least 90 min under the conditions described.

### *Quantification of cutaneous infiltrates*

In order to verify that elastase can be used as a marker for cutaneous neutrophils *in vivo*, we took advantage of the fact that topical application of $LTB_4$ to the skin induces a rapid and specific infiltrate of these cells (Nicholas *et al.*, 1983; Camp *et al.*, 1984; van de Kerkhof, Bauer & Maassen-de Groot, 1985).

Three volunteers were selected with no sign or history of skin disease. Aliquots of 1, 2, 5, 10 and 20 ng $LTB_4$ in ethanol were applied to test sites on the back as described previously (van de Kerkhof *et al.*, 1985). After 24 h, 'shave' biopsies were taken freehand using a razorblade in conjunction with a metal guard. The biopsies (approximately 3 mm diameter and 0·2 mm thick) were rinsed thoroughly in phosphate-buffered saline, dried between filter papers and weighed (average 1–2 mg). They were homogenized at 2 mg/ml in centrimide buffer using an all-glass Potter-type grinder, and the homogenate centrifuged (3000 *g* for 10 min) to yield a clear supernatant. Aliquots of 20 $\mu$l of this extract (diluted 1:10 with cetrimide buffer where necessary) were assayed for elastase as described previously.

Prior consent of the University Medical Ethics Committee was obtained for this experiment.

## RESULTS

### *Investigations* in vitro

The relationship between the fluorescence intensity and cell number for a lysate of blood-

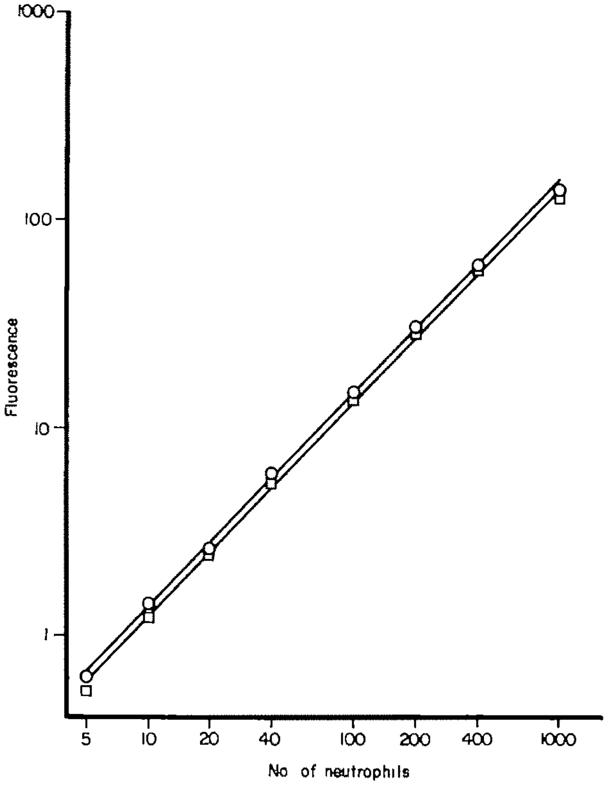

FIGURE 1. Log-log plot of fluorescence intensity (arbitrary units) against number of neutrophils per assay. (○) Neutrophils only; (□) neutrophils in the presence of 2 mg/ml skin extract. Correlation coefficient for each line > 0·99995.

derived neutrophils is illustrated in Figure 1 (upper line). It can be seen that as few as five cells per assay are sufficient for a reliable elastase measurement; this number yields a fluorescence of about double the blank value. The mean activity of three separate preparations (± SEM) was 0·57 ± 0·08 pmol/h/neutrophil.

Possible inhibition of neutrophil elastase activity by skin was studied by the assay of appropriate dilutions of the same neutrophil preparation in the presence of an extract of human skin (final concentration 2 mg/ml). The results are shown in Figure 1 (lower line). It can be seen that there is a slight inhibition (in this case 11%) which is independent of cell number. The mean inhibition produced by five different samples of normal human skin (all at 2 mg/ml) was 12% (SEM ± 4%).

*Quantification of cutaneous infiltrates*

No erythema, oedema or pustulation was visible 24 h after the application of $LTB_4$ in doses up to 20 ng per test site.

Elastase activity was determined on each biopsy, and the density of infiltrating neutrophils calculated using the value of 0·57 pmol/h/neutrophil (above). The results (mean ± SEM) are

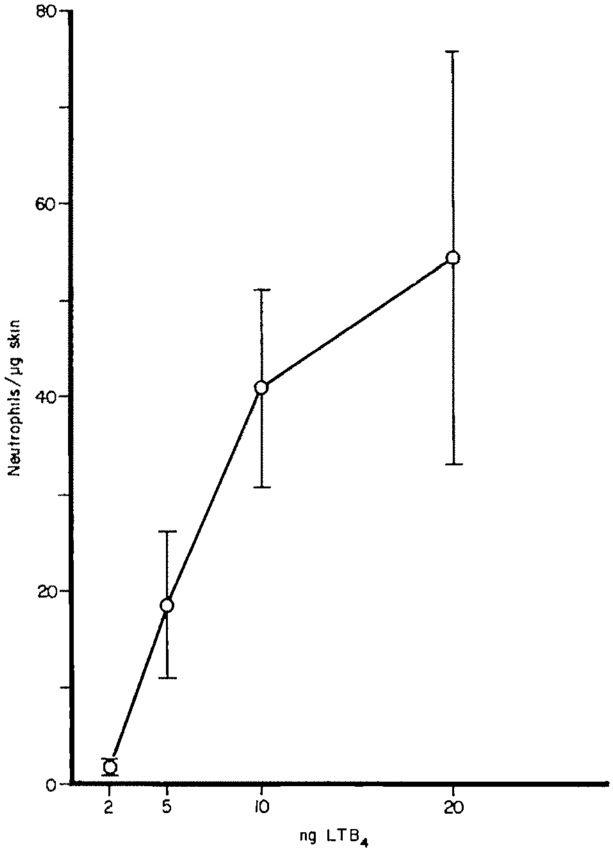

FIGURE 2. Quantification of neutrophil density in skin biopsies taken 24 h after different doses of $LTB_4$. Each point is the mean ± SEM of three experiments.

illustrated in Figure 2. It can be seen that a response could be measured in all three subjects down to 2 ng $LTB_4$, especially at the highest dose, however, there was a rather wide variation in the density of the infiltrate between the different subjects. No elastase activity was found in untreated skin, or in control sites treated with ethanol only or with a dose of 1 ng $LTB_4$.

## DISCUSSION

Using figures for the specific activity of purified leukocyte elastase against MeOSuc-Ala-Ala-Pro-Val-N-methylcoumarin quoted by Castillo *et al.* (1979) and correcting for the different substrate concentrations, the elastase activity reported here corresponds to about 0·6 pg enzyme protein per neutrophil. This compares well with the value of about 0·5 pg per neutrophil found by these authors. They estimated the ultimate sensitivity of the assay to be about 330 pg per ml elastase protein; for a 20 µl sample this would correspond to 13 neutrophils, again of the same order as in the present study. Thus, it is clear that the fluorometric determination of elastase as a marker for neutrophils is about a thousand times more sensitive than the spectrophotometric assay of myeloperoxidase, which requires a minimum of 10 000 cells per assay (Bradley *et al.*, 1982). This improvement in sensitivity permits the quantification of minimal infiltrates in very small biopsy specimens.

Elastase seems to be a rather specific marker enzyme for polymorphonuclear cells. The only other blood-derived cell possessing this enzyme is the monocyte, which contains about 5% of the activity found in the neutrophil (Davies *et al.*, 1984). Quantification of elastase in the eosinophil has not been reported, so care is required in the interpretation of data from infiltrates containing appreciable numbers of this cell type. Regarding the recovery of the extraction technique, it has been established that the elastase activity which is released following stimulation of the intact cell is 90–100% of that found after detergent lysis (Sklar *et al.*, 1982). The relatively small degree of inhibition of neutrophil elastase by skin extracts which we report here (averaging 12%) is presumably because the sulphated glycosaminoglycans, which are potent inhibitors of this enzyme (Menninger *et al.*, 1981), are complexed by centrimide (Scott, 1968). We found that replacing cetrimide by Triton X-100 as the detergent, although yielding the same elastase activity for blood-derived neutrophils, resulted in almost complete inhibition of the enzyme by skin extracts (data not shown). Nevertheless, it is clearly advisable to correct for endogenous inhibitors by the inclusion of a standardized neutrophil extract as an 'internal standard' in parallel assays.

The application of this approach to the *in vivo* situation has been demonstrated using $LTB_4$ as chemotactic agent. It is apparent that neutrophils can be quantified well below the level at which histological evidence of microabcessess has been reported (van de Kerkhof *et al.*, 1985).

The actual density of the infiltrate at the highest dose of $LTB_4$ (54 neutrophils per μg skin) is rather close to the figure of 85·5 neutrophils per μg skin reported in experimental staphylococcal infection of rat skin (Bradley *et al.*, 1982). There remains the possibility, however, that in chronic lesions much of the elastase may be released into the surrounding tissue and lost by diffusion or inactivation; the magnitude of this problem remains to be evaluated.

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# Immunohistochemical study of lysozyme in lupus miliaris disseminatus faciei

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## SUMMARY

The lysozyme activity in tissue samples from patients with lupus miliaris disseminatus faciei (LMDF), sarcoidosis and foreign body granuloma was investigated using the immunoperoxidase technique. The majority of epithelioid cells and giant cells in LMDF and sarcoidosis showed strong lysozyme staining in their cytoplasm. However, most macrophages and giant cells in foreign body granulomas, including granulomatous reactions to epidermal cysts and other foreign materials, stained weakly for lysozyme or were negative.

These results suggest that LMDF is different from the foreign body reaction to inert substances, and may be induced by an immunological mechanism associated with cell-mediated immunity.

Lysozyme, which is a bacteriolytic enzyme, has been found in myeloid cells, monocytes and histiocytes, and in various human secretions, by using the immunoperoxidase technique (Klockars & Reitamo, 1975; Mason & Taylor, 1975). Epithelioid and giant cells of immunological granuloma, such as tuberuculosis and sarcoidosis, show strong staining for lysozyme, compared with macrophages of foreign body granulomas (Yamashita, Iwamoto & Iijima, 1978; Blennerhassett & Papadimitriou, 1981).

Lupus miliaris disseminatus faciei (LMDF), alternatively called acne agnimata, shows histologically a tuberculoid structure with or without central caseation and is regarded as a manifestation of the papular type of acne rosacea (Lever & Schaumburg-Lever, 1975). However, the histopathogenesis of this disorder is still unknown.

In the present study, we examined the lysozyme activity of epithelioid cells and giant cells in LMDF using the immunoperoxidase technique, and compared the staining pattern with that in sarcoidosis and foreign body granuloma to determine whether this epithelioid cell granuloma is related to an immunological reaction.

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## METHODS

Formalin-fixed, paraffin-embedded tissues from seven cases of LMDF, three cases of sarcoidosis, and five cases of foreign body granulomas were studied. Clinical and pathological features of the cases are summarized in Table 1. The tissue sections, cut at 5 μm, were dewaxed in xylene, and rehydrated through graded alcohols for 30 min. The immunoperoxidase procedure was carried out as described previously (Mason & Taylor, 1975). Briefly, after incubation in absolute methanol containing 0·3% hydrogen peroxidase for 30 min, the sections were sequentially incubated with rabbit anti-human-lysozyme antiserum 1/100 (Dakopatts, Denmark), and swine anti-rabbit serum IgG conjugated with horseradish peroxidase 1/20 (Dakopatts, Denmark). Phosphate buffered saline (PBS) (0·1 M, pH 7·4) was used for washing and dilutions. Subsequently, the sections were treated with freshly prepared 3-,3′-diaminobenzidine solution with hydrogen peroxide at room temperature, and counterstained with haematoxylin. Controls included the substitution of normal rabbit serum (Dakopatts, Denmark) for the anti-lysozyme antiserum.

## RESULTS

The results of immunoperoxidase staining are summarized in Table 1. In all seven cases of LMDF, epithelioid cells and giant cells showed moderate to intense staining for lysozyme, which appeared as granular intracytoplasmic deposits (Figs 1 and 2). In all three cases of

TABLE 1. Clinical and pathological features and results of immunostaining for lysozyme in LMDF, sarcoidosis and foreign-body granuloma

| | Patient no. | Sex | Age (years) | Site of papules or nodules | Histology** | Lysozyme activity* Epithelioid cells or macrophages | Lysozyme activity* Giant cells |
|---|---|---|---|---|---|---|---|
| LMDF | 1 | M | 43 | Eyelids | Caseation − | + | ++ |
| | 2 | F | 57 | Face | Caseation + | ++ | ++ |
| | 3 | M | 43 | Face | Caseation + | ++ | ++ |
| | 4 | F | 51 | Eyelids | Caseation − | ++ | ++ |
| | 5 | F | 59 | Eyelids | Caseation + | + | ++ |
| | 6 | M | 36 | Perioral area | Caseation − | ++ | ++ |
| | 7 | F | 37 | Eyelids | Caseation − | + | ++ |
| Sarcoidosis | 8 | F | 70 | Nose | | ++ | ++ |
| | 9 | F | 57 | Right lower leg | | + | ++ |
| | 10 | F | 28 | Right knee | | ++ | ++ |
| Foreign-body granuloma | 11 | M | 54 | Right thigh | Suture material | − | ± |
| | 12 | F | 18 | Nose | Cyst | − | − |
| | 13 | M | 2 months | Right upper arm | Spicule of cactus | − | − |
| | 14 | M | 31 | Penis | Lipid | − | ± |
| | 15 | F | 48 | Left corner of the mouth | Ruptured cyst | − | ± |

* ++: intensely positive, +: positive, ±: faintly positive, −: negative.
** Caseation, +: present. −: absent.

sarcoidosis, epithelioid cells and giant cells also showed similar positive staining for lysozyme (Figs 3 and 4). In foreign body granulomas, giant cells were faintly positive in some cases; however, most giant cells and most macrophages were negative (Figs 5 and 6). All the controls were negative.

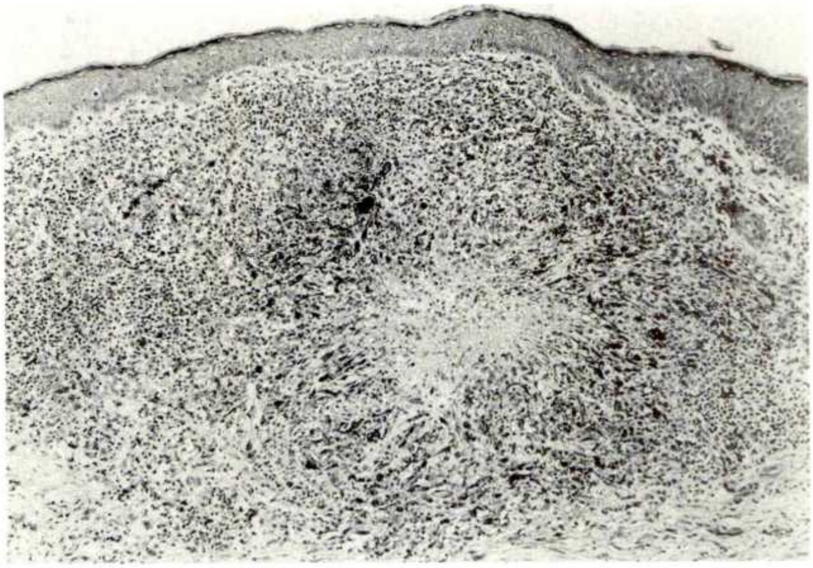

FIGURE 1. LMDF. Lysozyme-positive epithelioid cells and giant cells around caseation (Case 2) (Original × 90).

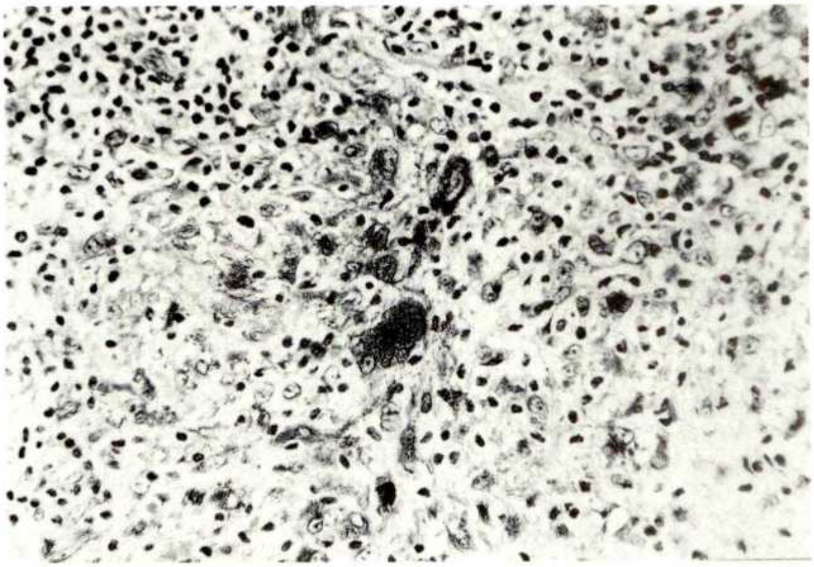

FIGURE 2. LMDF. Higher magnification of Figure 1 showing intensely granular intracytoplasmic lysozyme activity in the epithelioid cells and giant cells (Case 2) (Original × 360).

## DISCUSSION

In the present study, the epithelioid and giant cells in LMDF and sarcoidosis showed a strong staining reaction for lysozyme, but foreign body granulomas had very little lysozyme activity. Similar results in sarcoidosis and foreign body granuloma have been reported previously

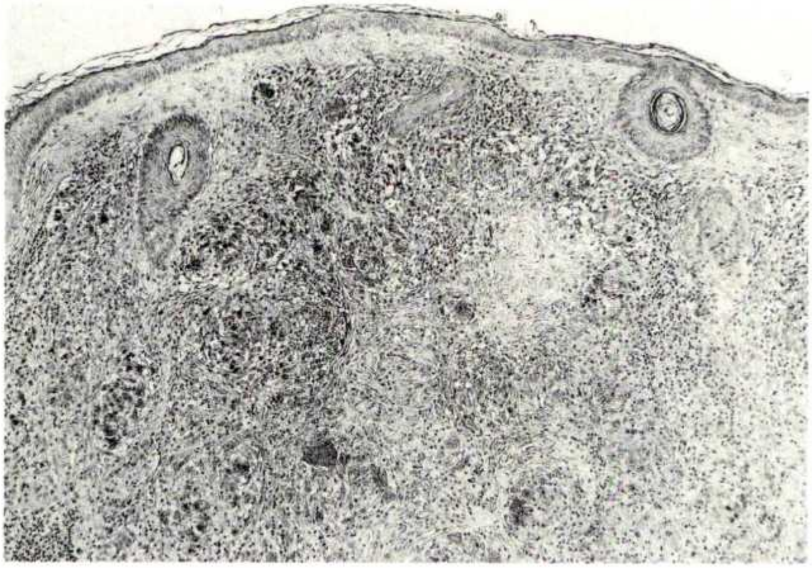

FIGURE 3. Sarcoidosis. Lysozyme-positive epithelioid cells and giant cells (Case 10) (Original × 87).

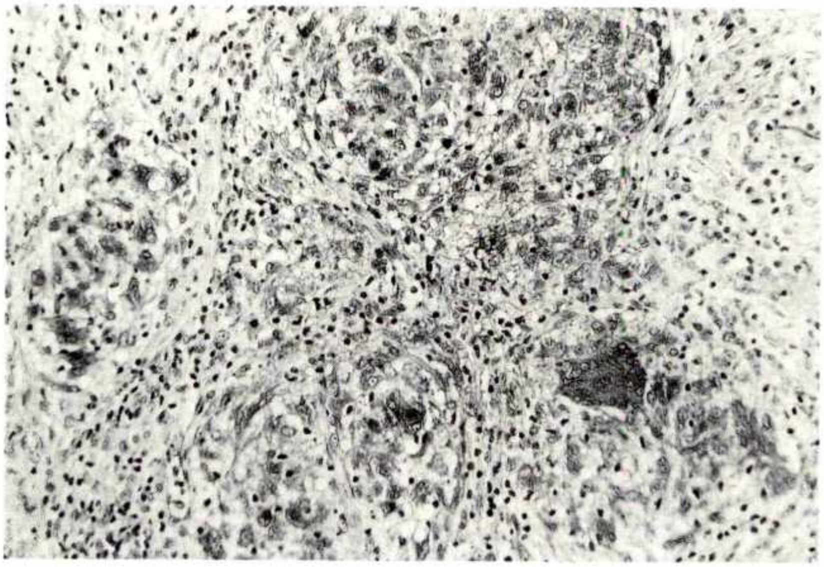

FIGURE 4. Sarcoidosis. Higher magnification of Figure 3 showing intensely granular intracytoplasmic lysozyme activity in the epithelioid cells and giant cells (Case 10) (Original × 220).

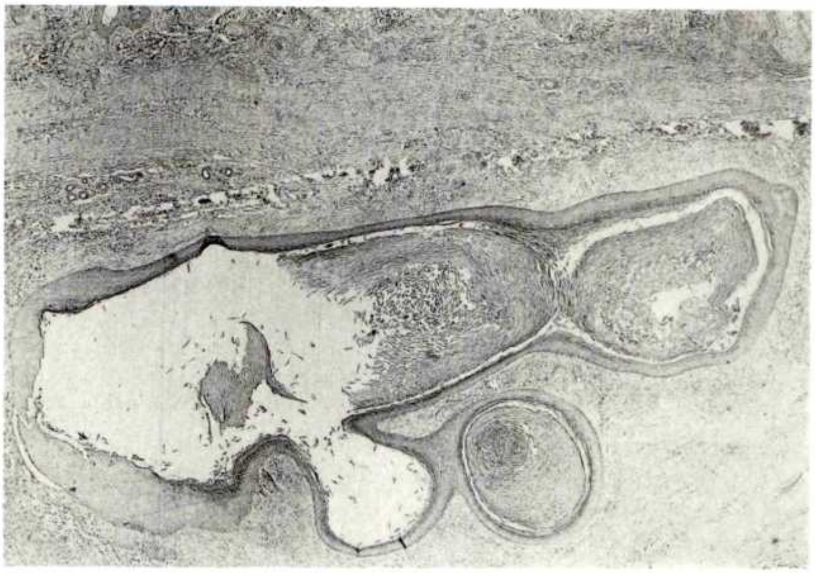

FIGURE 5. Foreign body granuloma. No staining of the cellular infiltrate for lysozyme (Case 12) (Original × 30).

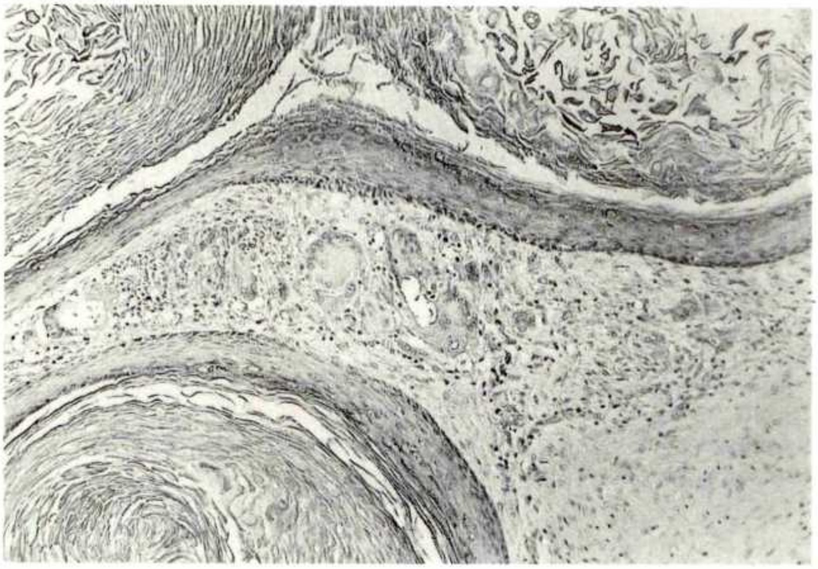

FIGURE 6. Foreign body granuloma. Higher magnification of Figure 5 showing no staining of macrophages and giant cells for lysozyme (Case 12) (Original × 116).

(Yamashita *et al.*, 1978; Blennerhassett & Papadimitriou, 1981). Foreign body granuloma represents a non-immunological reaction of mononuclear macrophages to an insoluble foreign material and epithelioid cell granuloma is seen as a further development of foreign body granuloma (Adams, 1976).

The epithelioid cell is derived from the monocyte as a purely secretory activated macrophage which has essentially a degradative function. The lysozyme activity in LMDF may possibly occur at a particular stage of macrophage activation. Lysozyme is considered to be a constituent of primitive non-specific defence mechanisms, especially against infection. Various infectious agents, including parasites, deep mycoses, mycobacteria, and some other micro-organisms, induce cell-mediated and humoral immunity and cause the formation of epithelioid cell granulomas. Sarcoidosis has been considered to be a cell-mediated granuloma as occurs in tuberculosis and fungal infection (Warren, 1976). The finding of an intense lysozyme reaction in LMDF suggests that the disease is induced by some unknown infectious agent associated with cell-mediated immunity. Daems, Koerten and Soranzo (1976) showed a distinct difference histochemically between monocyte-derived and tissue-derived macrophages. This allows an alternative interpretation of the different staining for lysozyme in our study, namely that the enzyme activity in LMDF is only found at certain stages of histiocyte differentiation and possibly only in certain subsets. Recently, LMDF has been explained as a foreign body reaction against sebum and keratinized cells from disintegrating hair structures (Ueki & Masuda, 1979; Pinkus & Mehregan, 1981). We suggest that LMDF is a reaction to an infectious agent, associated with cell-mediated immunity. Grosshans, Kremer and Maleville (1974) regarded this granulomatous formation as a delayed type hypersensitivity reaction to the mite *Demodex folliculorum*. We did not identify an aetiological agent in the present study.

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# Hyper-induction of skin ornithine decarboxylase activity in psoriatic patients

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## SUMMARY

Ornithine decarboxylase activity was measured in skin from 14 normal and 24 psoriatic subjects (with stable plaque type psoriasis vulgaris) using punch biopsies carried out immediately (0 h) and 6 h after stimulation by cellotape stripping. At 0 h, no significant difference was found between normal and involved or uninvolved psoriatic skin, but a significant increase was measured 6 h after tape stripping. This increase was significantly greater in uninvolved and involved psoriatic skin compared with normal skin. Preliminary results indicated that these changes were localized in the epidermis.

Biosynthesis and accumulation of the polyamines putrescine, spermidine and spermine are associated intimately with cellular growth and division (Pegg & McCann, 1982; Lowe, 1980). In the involved and uninvolved skin of psoriatic patients there is a more rapid epidermal cell proliferation than in normal skin as measured by epidermal DNA synthesis (Goodwin, Hamiltion & Fry, 1973). Nevertheless, the specific role of polyamines in the aetiopathology of psoriasis is still debated (McDonald, 1983; Voorhees, 1979; Grosshans *et al.*, 1980). Although it has been established clearly that polyamine concentration and basal activity of the rate-limiting polyamine biosynthetic enzyme, ornithine decarboxylase (ODC) (EC:4.1.1.17) are elevated in involved skin, an abnormality of polyamine metabolism in uninvolved skin is still controversial (Russell *et al.*, 1978; Lowe, Breeding & Russell, 1982; Lauharanta *et al.*, 1981; Bohlen *et al.*, 1978; Proctor *et al.*, 1979).

It is difficult to measure small variations in enzyme activity because basal activity of ODC in the skin is very low relative to the sensitivity of the assay method. In animal skin, ODC activity can be induced markedly by stimuli which increase cell proliferation, TPA (12-o-tetradecanoyl-phorbol-13-acetate) application (O'Brien, Simisiman & Boutwell, 1975), ultraviolet irradiation (Lowe & Breeding, 1982) or cellotape stripping (Connor & Lowe, 1983; Bouclier *et al.*, 1984).

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In the present study we used cellotape stripping to induce ODC activity in the skin of healthy volunteers and psoriatic patients.

## METHODS

### *Selection of subjects*

ODC activity was measured in full thickness skin biopsies from 12 normal volunteers (three women and nine men, aged 32–80 years) and 21 psoriatic patients (stable plaque type psoriasis vulgaris, six women and 15 men, aged 18–68 years). In psoriatic subjects, both involved and uninvolved skin were tested. In addition, skin from three normal volunteers and three psoriatic patients was used to measure ODC activity in the epidermis and in the dermis. Individuals using oral or topical medication during the 2 weeks prior to the study were excluded. All skin samples were taken from the abdomen, from an area that had not been exposed to sunlight or phototherapy.

### *Stripping procedure*

Skin was stripped by successive application of cellotape. Removal of the stratum corneum was considered to be complete when the whole area appeared to be glistening (40 applications of cellotape; van de Kerkhof *et al.*, 1983). No difference was observed between patients and controls.

### *Histological studies*

Biopsies of 6 mm taken from normal and stripped skin were studied histologically using paraffin embedded sections (5 $\mu$m) stained with haematoxylin phloxin safran (HPS).

### *Measurement of skin ODC activity*

Punch biopsies of 6 mm diameter were taken immediately (0 h) and 6 h after cellotape stripping, and stored in liquid nitrogen. ODC activity was measured the following day.

The biopsies were homogenized in 1 ml sodium phosphate buffer 50 mM, pH 7 containing 0·1 mM EDTA, 5 mM dithiothreitol (DTT) and 0·1 mM pyridoxal phosphate (PLP) using a Polytron homogenizer. The homogenates were centrifuged at 10 000 ***g*** for 5 min at 4°C and the supernatants were used for the enzyme assay.

The determination of ODC activity is based on the measurement of $^{14}CO_2$ liberated from L-1-$^{14}$C ornithine (Danzin *et al.*, 1979). The closed reaction vessel fitted with a filter paper soaked in 3 M hyamin hydroxide contained 1 ml of assay medium (50 mM phosphate buffer pH 7, 5 mM DTT, 0·1 mM PLP, 0·1 mM L-ornithine, 1·25 $\mu$Ci L-1-$^{14}$C ornithine and the skin sample).

After incubation at 37°C for 1 h, the reaction was stopped by the addition of 500 $\mu$l of 40% trichloroacetic acid. The $^{14}CO_2$ trapped on the filter paper was counted in a standard scintillation fluid. The enzyme activity was calculated as nmoles of $CO_2$ liberated/h/mg protein. Protein content was determined using the Bio-Rad assay.

### *Measurement of ODC activity in epidermis and dermis*

Punch biopsies of 6 mm diameter taken at 0 h and 6 h after cellotape stripping and stored in liquid nitrogen for one night were used. Vertical and horizontal sections were cut from the frozen biopsies using a cryostat. The epidermal thickness of each biopsy was measured using a 10 $\mu$m vertical section stained with toluidin blue.

The epidermis was separated from the dermis by an horizontal section. The thickness of this section was determined by the measured thickness of the epidermis (about 50–70 μm for normal skin). We confirmed that all the epidermis was removed from the dermis by microscopic examination. The epidermal samples obtained thus were relatively pure.

ODC activity was measured in the samples of epidermis and dermis as described above.

### *Statistical analysis*

Enzyme activities at 0 h and 6 h were compared using a paired *t*-test. Other comparisons were made using a Mann-Whitney U test.

## RESULTS

Figure 1 shows that cellotape stripping produced by 40 successive applications of cellotape resulted in complete removal of the stratum corneum. No alteration of the other epidermal layers was observed.

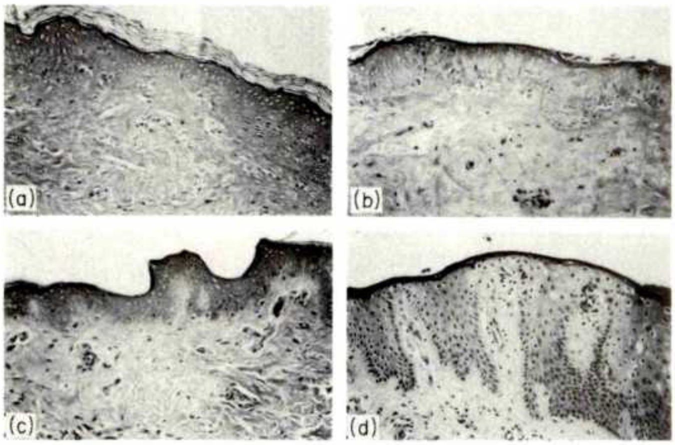

FIGURE 1. Removal of the stratum corneum by cellotape stripping; (a) normal skin; (b) stripped normal skin; (c) stripped uninvolved psoriatic skin; (d) stripped involved psoriatic skin.

We examined normal and uninvolved and involved psoriatic skin, just after cellotape stripping (0 h) and 6 h later. As shown in Figure 2, the enzyme activities were not significantly different at 0 h (0·06–0·08 nmol/h/mg protein) but they are significantly elevated 6 h after stimulation by tape stripping. The measured activity was 0·14 ± 0·04, 0·36 ± 0·11, 0·41 ± 0·09 nmol/h/mg protein (mean ± SEM) in normal skin ($P < 0{\cdot}05$) and uninvolved and involved psoriatic skin respectively ($P < 0{\cdot}005$).

The induced ODC activity at 6 h was significantly greater in both uninvolved and involved psoriatic skin compared with normal skin ($P < 0{\cdot}005$).

ODC activity in epidermis and dermis from three normal volunteers and three patients is shown in Table 1. In the dermis, activity was low and no difference was observed between normal and uninvolved psoriatic dermis at 0 h, and no change was induced by cellotape stripping (6 h). Activity in the epidermis measured at 0 h was no different in normal and uninvolved psoriatic epidermis. At 6 h, ODC activity was increased. Induced activity was greater in uninvolved psoriatic epidermis than in normal epidermis.

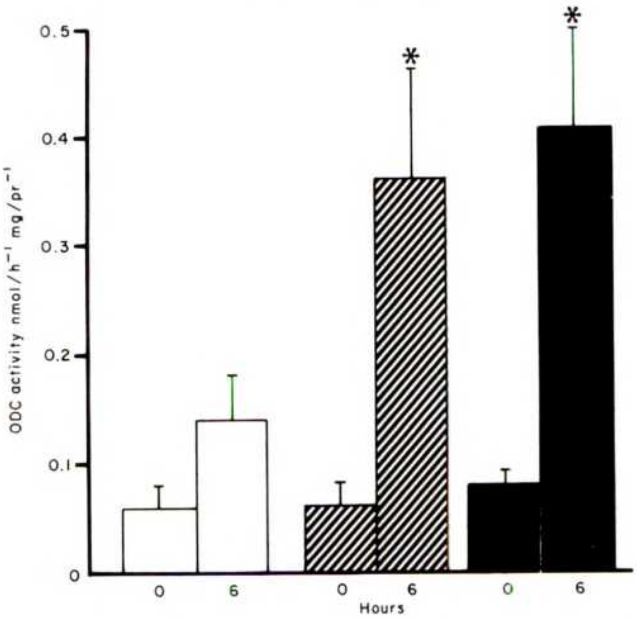

FIGURE 2. Induction of ornithine decarboxylase (ODC) activity in human skin by cellotape stripping. Columns give mean and SEM; □ normal skin ($n=11$); ▨ uninvolved psoriatic skin ($n=21$); ■ involved psoriatic skin ($n=21$); measured immediately after tape stripping (0) and 6 h later. *Comparison of activity at 6 h in normal and psoriatic skin. $P<0{\cdot}005$, Mann-Whitney U test.

TABLE 1. Ornithine decarboxylase (ODC) activity in human epidermis and dermis after cellotape stripping

| | ODC activity nmol/h/mg protein* | | | |
|---|---|---|---|---|
| | Epidermis | | Dermis | |
| Time after cellotape stripping (h) | 0 | 6 | 0 | 6 |
| Normal skin | 0·14 ± 0·02 | 0·24 ± 0·03 | 0·08 ± 0·03 | 0·05 ± 0·02 |
| Uninvolved psoriatic skin | 0·17 ± 0·07 | 1·3 ± 0·4 | 0·09 ± 0·04 | 0·06 ± 0·03 |

* Values are means ± SEM for three subjects.

## DISCUSSION

Very low or undetectable ODC activity has been reported previously in two studies in normal human epidermis (Russel *et al.*, 1978; Lowe *et al.*, 1982), and these authors did not observe a significant difference in uninvolved psoriatic epidermis. In our study, we observed no significant differences between ODC activity measured at 0 h (just after tape stripping) in normal and involved and uninvolved psoriatic skin, or between normal and uninvolved psoriatic epidermis and dermis. It is possible that the tape stripping procedure can modify the expression of the ODC activity measured immediately afterwards and mask small variations in enzyme activity.

Although there are several reports of the induction of ODC activity in mouse skin, induction of ODC activity in human skin has not been documented previously to our knowledge. In the present work, we have shown that in human skin, the ODC activity measured after tape stripping was significantly increased. This result shows that cellotape stripping of human skin is also able to induce ODC, as has been reported previously in mouse and rat skin (Connor & Lowe, 1983; Bouclier *et al.*, 1984). In the psoriatic patient, the increase in ODC activity 6 h after stimulation was significantly greater than in normal individuals. Interestingly, the same increase was obtained with both involved and uninvolved skin. Thus, there is probably a basic defect of polyamine biosynthetic activity in the uninvolved skin which is not apparent in unstimulated skin. This defect is probably localized in the epidermis. Our preliminary results (Table 1) indicated that ODC activity in dermis was low and was not modified by cellotape stripping. Only epidermal enzyme activity was increased; the activity measured 6 h after stimulation was about 5-fold higher in uninvolved psoriatic epidermis than in normal epidermis. A larger number of individuals need to be tested to confirm this finding.

Numerous studies have demonstrated an imbalance in a variety of intracellular mediators and related biochemical systems in psoriatic skin (Marcelo & Voorhees, 1983). The epidermal abnormality is not restricted to the lesional area; the clinically normal skin of the psoriatic patient exhibits a number of anomalies when compared with normal skin, an increase in DNA synthesis (Marks, 1978), a raised level of phospholipase $A_2$ activity (Verhagen *et al.*, 1984), decreased levels of histidine and urocanic acid (Caron *et al.*, 1983) and an increase in polyamine content (Russel *et al.*, 1978).

The hyperinduction of ODC activity demonstrated in our study may reflect an abnormal predisposition of uninvolved psoriatic skin to proliferate more rapidly after a stimulus. This would be in agreement with the work of Wiley and Weinstein (1979) who showed that uninvolved psoriatic skin has a greater proliferative response after tape stripping than does normal skin. Clinically, this would be expressed as the Koebner phenomenon. In our study, however, we did not observe an induction of psoriatic lesions on the stripped area, but all the patients were treated after the experiment.

This study demonstrates an abnormality in the regulation of polyamine biosynthesis in uninvolved and involved psoriatic skin, which is clearly apparent after stimulation by tape stripping.

## ACKNOWLEDGMENTS

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# The autoimmune background in lichen planus

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## SUMMARY

Fifty-four patients with lichen planus (LP) and 54 age- and sex-matched controls were studied. No increase in the incidence of autoimmune disease was found in the patients with LP or their relatives. There was no increase in the frequency of autoantibodies in the LP group and serum immunoglobulin levels were not altered significantly. In the majority of cases, LP is not part of a generalized autoimmune disturbance.

Abnormal immune mechanisms are thought to be important in the pathogenesis of lichen planus (LP) (Black, 1977) and there have been a number of reports of the association of LP with various autoimmune disorders, most notably primary biliary cirrhosis (Graham-Brown, Sarkany & Sherlock, 1982; Powell, Rogers & Dickson, 1982). Some authors have found abnormal immunoglobulin levels in patients with LP (Lehner, 1969; Stankler, 1975; Jacyk & Greenwood, 1978; Mahood, 1981), while others have not (Griffith, Kaufman & Silverman, 1974; Scully, 1982). We therefore undertook a systematic study of 54 patients with histologically-proven LP to determine the incidence of autoimmune disorders and autoantibodies, and to examine the levels of serum immunoglobulins.

## METHODS

### *Patients*

Fifty-four consecutive patients (30 females, 24 males) who presented to the Department of Dermatology at the Leicester Royal Infirmary with a diagnosis of LP took part in the study. Histological confirmation of the clinical diagnosis was obtained in all cases. A control sample, matched for age and sex, was recruited from patients concurrently attending the Dermatology Clinic with other conditions. Patients with dermatoses known or suspected to be immunologically modulated (e.g. eczema, psoriasis, and alopecia areata) were specifically excluded from the control group. The majority of patients in the control group had benign skin tumours or warts.

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A full clinical history was obtained from each patient, with particular reference to preceding or co-existing autoimmune disease. A family history of autoimmune disease was also specifically sought.

### *Serum immunoglobulins*

Serum levels of IgG, IgA and IgM were estimated immunochemically using a Hyland laser nephelometer.

### *Autoantibodies*

Sera were screened (at a 1:20 dilution) for antinuclear, smooth muscle, mitochondrial and gastric parietal cell antibodies by indirect immunofluorescence on cryostat sections of rat liver, rat kidney and mouse stomach. Positive sera were titrated. Sera containing gastric parietal cell antibodies were tested for intrinsic factor antibodies against $^{57}$Co-B12 by a competitive binding radioimmunoassay. Thyroid microsomal and thyroglobulin antibodies were detected and titrated by haemagglutination of antigen-coated turkey erythrocytes (Thymune-M and -T kits Wellcome).

## RESULTS

The age range of the patients was 13–81 years (mean 45 years). Of the 54 patients with LP, three (6%) had oral involvement only, 12 (22%) had both oral and cutaneous lesions, and 39 (72%) had cutaneous lesions only.

### *Serum immunoglobulins*

Serum levels of IgG, IgA and IgM are presented in Figure 1. Two patients with LP had selective IgA deficiency, and four other patients had low IgM levels, compared with none in the control group. However, analysis of logarithmically-transformed data using a paired *t*-test failed to show any statistically significant difference in the levels of these immunoglobulins between the LP group and the controls.

### *Autoimmune disease*

Autoimmune disease was found in four (7%) patients in the LP group (pernicious anaemia in two cases, primary biliary cirrhosis in one case and alopecia areata in one case) compared with two (3·5%) patients in the control group (both with pernicious anaemia) (Table 1). This difference is not statistically significant, using the binomial test. Three patients in the LP group had first or second degree relatives with a history of autoimmune disease (two with pernicious anaemia, one with thyrotoxicosis) compared to none in the control group. This difference was also not statistically significant (binomial test).

### *Autoantibodies*

Twenty (37%) patients in the LP group were found to have titratable levels of autoantibodies compared with 21 (39%) patients in the control group (Table 1). No particular antibody was found more frequently in the LP group. We have therefore been unable to show an association between the presence of LP and the incidence of circulating autoantibodies.

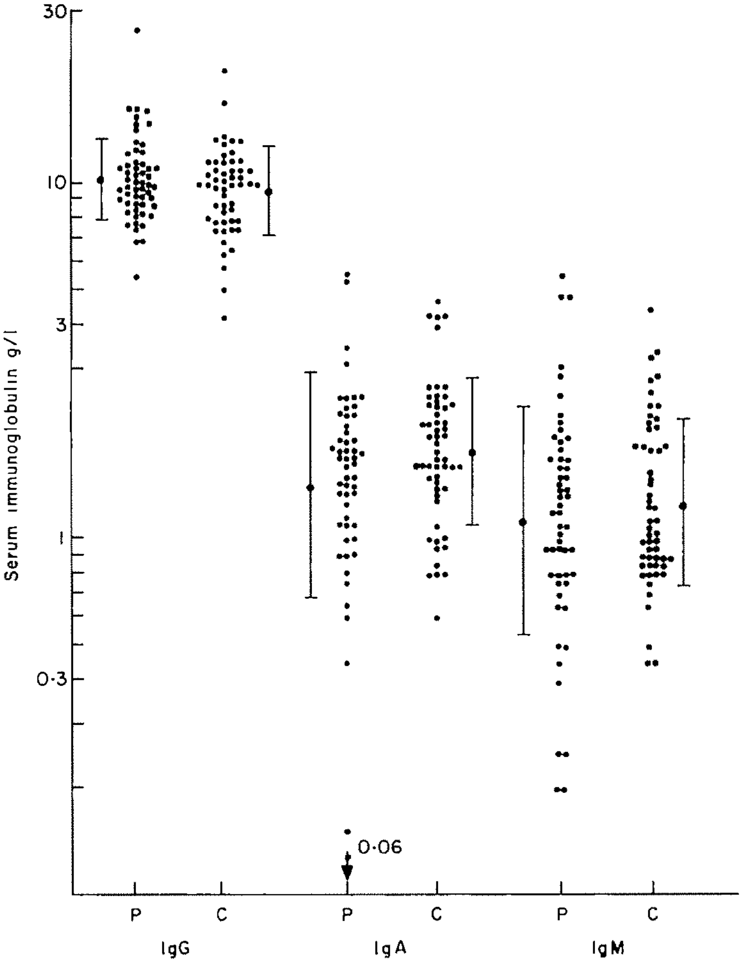

FIGURE 1. Serum IgG, IgA and IgM levels in lichen planus patients (P) and controls (C). Vertical bars indicate logarithmic means ± 1 s.d.

TABLE 1. Autoimmune disease and autoantibodies in 54 patients with LP and matched controls

| | No. of patients | No. of controls |
|---|---|---|
| Autoimmune disease | 4 | 2 |
| Pernicious anaemia | 2 | 2 |
| Primary biliary cirrhosis | 1 | 0 |
| Alopecia areata | 1 | 0 |
| Autoantibodies | 20 | 21 |
| Antinuclear | 2 | 5 |
| Smooth muscle | 6 | 6 |
| Mitochrondrial | 1 | 1 |
| Gastric parietal cell | 6 | 5 |
| Intrinsic factor | 2 | 2 |
| Thyroid microsomal | 7 | 9 |
| Thyroglobulin | 2 | 2 |

## DISCUSSION

Although lichen planus is a common disorder, the aetiology remains obscure. It is thought that abnormalities of immune function may play a role in the pathogenesis of the skin lesions (Black, 1977) and immunofluorescent and immunohistochemical findings support this. Altered levels of circulating immunoglobulins have been found by some investigators (Lehner, 1969; Stankler, 1975; Jacyk & Greenwood, 1978; Mahood, 1981), although others have found no abnormality (Griffith *et al.*, 1974; Scully, 1982). The results of previous studies are summarized in Table 2. There are also a number of case reports of the association of lichen planus with various conditions in which abnormal immune function is of importance, including ulcerative colitis and myasthenia gravis (Miller, 1971), alopecia areata (Tan, 1974) and primary biliary cirrhosis (Graham-Brown *et al.*, 1982; Powell *et al.*, 1982). Lichen planus-like lesions are a frequent occurrence in early chronic graft-versus-host disease (Lerner *et al.*, 1974). These observations have been used to lend support to an 'autoimmune' pathogenesis for lichen planus.

TABLE 2. Reports of immunoglobulin levels in lichen planus

| Reference | IgG | IgA | IgM | No. of patients studied |
|---|---|---|---|---|
| Lehner, 1969 | Decreased | — | Normal | 20 |
| Griffith *et al.*, 1974 | Normal | — | — | 35 |
| Stankler, 1975 | Normal | Decreased | Decreased | 13 |
| Jacyk and Greenwood, 1978 | Normal | Normal | Decreased | 42 |
| Mahood, 1981 | Increased | Increased | Increased | 31 |
| Scully, 1982 | Normal | Normal | Normal | 35 |

Despite this, there does not appear to have been any systematic study to determine the incidence of autoimmune disease in an unselected group of patients suffering from lichen planus. Furthermore, if there were an autoimmune background to lichen planus, it might be anticipated that auto-antibodies would be found with increased frequency when compared with a control group, as appears to be the case, for example, in lichen sclerosus et atrophicus (Harrington & Dunsmore, 1981).

The results of our study showed no significant difference in immunoglobulin levels between the lichen planus group and the control group, although a few lichen planus patients had low IgA or IgM levels. We were also unable to demonstrate any increase in the prevalence of either organ-specific or non-organ-specific autoantibodies in the lichen planus group; in fact, autoantibodies were found more frequently in the control group. The incidence of autoimmune disease was not significantly increased in the lichen planus group or in their relatives.

This study shows that our patients presenting with lichen planus did not have any specific abnormality of immunoglobulin production nor did there appear to be a relationship with autoimmune disease.

Our study was not designed to demonstrate an increased incidence of lichen planus in groups of patients suffering from specific autoimmune diseases (e.g. primary biliary cirrhosis) and it may be that in these cases specific abnormalities of immune function are of importance in the development of both conditions. However, the present results suggest that, in the majority of cases, lichen planus is not part of a generalized autoimmune disturbance.

## ACKNOWLEDGMENT

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# Plasminogen activator secreted by cultured human melanocytes

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## SUMMARY

A plasminogen activator (PA), $M_r$ 72 000, was detected in conditioned medium from human melanocyte cultures by fibrin autography. The electrophoretic mobility was identical to that of tissue PA produced by Bowes melanoma cells. PA activity in human melanocyte culture medium was inhibited by anti-tissue PA IgG, but not by anti-urokinase IgG. Our results are the first to show that normal human melanocytes in culture secrete tissue plasminogen activator.

Plasminogen activators (PA) are serine proteinases which play a central role in the regulation of fibrinolysis. Recently, many biological processes have been proven to be associated with cellular secretion of PA. These processes include cell migration, tissue destruction and tissue remodelling (Reich, 1978). A wide variety of cells are reported to produce PA (Gronow & Bliem, 1983), including cultured human epidermal cells, and cultured human skin fibroblasts (Hashimoto *et al.*, 1983a; Scott *et al.*, 1983). An increase in PA activity has been described in several skin diseases, including psoriasis and pemphigus (Fraki, Briggaman & Lazarus, 1982; Hashimoto *et al.*, 1983b).

Melanoma cells secrete PA (Wilson, Becker & Hoal, 1980), but no studies have reported PA in normal melanocytes. Recent progress in cell culture techniques have made it possible to culture human melanocytes (Eisinger & Marko, 1983), and in the present study we have investigated whether human melanocytes in culture secrete plasminogen activators.

## METHODS

### *Cell culture*

Human melanocytes were isolated from newborn foreskin. Melanocytes were grown in minimum essential medium (MEM) with Earle's salts (Gibco Laboratories, Grand Island, NY,

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U.S.A), supplemented with 5% fetal bovine serum (FBS) (Flow Laboratories, Rockville, MD, U.S.A.), 10 ng/ml TPA (12-o-tetra-deconyl-phorbol-13-acetate) (Consolidated Midland Corp., Brewster, NY, U.S.A), and $10^{-8}$ M cholera toxin (Schwarz/Mann, Orangeburg, NY, U.S.A.), as reported previously (Eisinger & Marko, 1983; Horikoshi *et al.*, 1984). Melanocytes were identified by electron microscopy on the basis of the presence of pre-melanosomes and melanosomes. Contamination with fibroblasts and keratinocytes was excluded as follows. Fibroblasts were stained for leucine aminopeptide, an enzyme characteristic for fibroblasts. Keratinocytes were distinguished morphologically. Human melanoma cells (Bowes), provided by Dr D.B.Rifkin of New York University, were grown in Dulbecco's Modified Eagle's medium (DME) (Nissui, Tokyo, Japan) with 10% FBS.

Serum-free conditioned medium was prepared as follows. Cell monolayers in 35 mm dishes (Falcon, Oxnard, CA, U.S.A.) were washed twice with PBS and once with MEM and DME. Cells were then incubated for 24 h with serum-free medium, MEM with TPA for melanocytes and DME for melanoma cells. This conditioned medium was then removed, centrifuged and stored at −70°C. In the present study, conditioned media from three different cultures of melanocytes were used.

### *Fibrin autography*

Fibrin agar indicator gels were prepared according to the modified method of Granelli-Pipperno and Reich (1978). Briefly, a solution of agarose, plasminogen, thrombin and fibrinogen was mixed and poured onto a glass plate. Final concentrations were 1·3% agarose, 32 μg/ml plasminogen, 0·08 IU/ml thrombin, 2·25 mg/ml fibrinogen. The fibrin agar gel was used immediately. After SDS polyacrylamide gel electrophoresis using the buffer system described by Laemmli (1970), the SDS gels were soaked in 2·5% Triton X-100 for 1·5 h to remove SDS, patted dry with a paper towel, and applied to the surface of the fibrin agar indicator gel. The indicator gel was allowed to develop at 37°C in a moist chamber, and photographed. Dark areas on the indicator gel correspond to lytic zones, which indicate the presence of PA. No lytic zones were detected without plasminogen.

### *Purification of anti-urokinase and anti-tissue PA IgG*

The IgG fraction of rabbit anti-urokinase antibody (Mochida Pharmacy Co., Tokyo, Japan) was purified by protein A-Sepharose as described previously (Hashimoto *et al.*, 1983b). Goat anti-tissue PA antibody was provided by Dr D.B.Rifkin of New York University. An IgG fraction of this antibody was purified by ammonium sulphate precipitation and ion exchange chromatography (Garvey, Cremer & Sussdeorf, 1977). Purified IgG was dialysed against PBS and stored at −70°C.

### *Specificity of anti-urokinase IgG and anti-tissue PA IgG*

Urokinase standard supplied by Mochida Pharmacy Co. (Tokyo, Japan) was used. This urokinase contains no tissue PA. Tissue PA from Bowes melanoma cells was partially purified by concanavalin A-Sepharose. This partially purified tissue PA was used after absence of contamination with urokinase was confirmed by fibrin autography.

Urokinase (5 IU/ml) was mixed with an equal volume of anti-urokinase IgG at various concentrations. Urokinase activity of 2 μl of this mixture was checked by the fibrin plate method. At a concentration of 50 μg/ml, anti-urokinase IgG inhibited urokinase activity completely. Anti-tissue PA IgG had no effect on urokinase activity even at 500 μg/ml. Tissue PA (equivalent to 5 IU/ml of urokinase) was used in the same way. Anti-tissue PA IgG at 200 μg/ml inhibited tissue PA activity completely, However, anti-urokinase IgG at 500 μg/ml had no effect on tissue PA activity.

### $^{125}$*I-fibrin plate assay*

PA activity was assayed using multi-well culture dishes coated with $^{125}$I-fibrin as described previously (Hashimoto *et al.*, 1983b). The PA activity in conditioned medium from human melanocyte cultures was determined as follows. The conditioned medium was incubated with an equal volume of either anti-urokinase or anti-tissue PA IgG at various concentrations for 30 min on ice, and 50 μl of this mixture and plasminogen were added to the $^{125}$I-fibrin coated wells. The inhibition of $^{125}$I-fibrin release was expressed as a percentage of that in controls.

## RESULTS

As shown in Figure 1, the PA in conditioned medium from human melanocytes gave one lytic zone at $M_r$ 72 000. Its electrophoretic mobility was identical to that of tissue PA produced by Bowes melanoma cells. The PA secreted by cultured human melanotyes was examined to

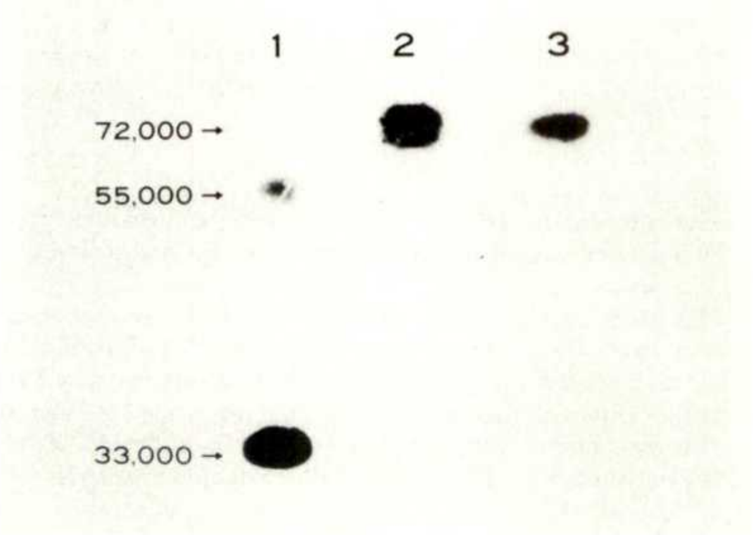

FIGURE 1. Identification of plasminogen activator in the conditioned medium from human melanocytes by fibrin autography. Lane 1: urokinase (50 mIU); Lane 2: tissue plasminogen activator from the culture medium of Bowes melanoma cells (50 mIU of urokinase equivalent activity); Lane 3: conditioned medium from human melanocytes (50 μl). Molecular weights were calculated from the position of markers (Bio-Rad, low molecular weight standards) electrophoresed in parallel lanes and stained with Coomassie Brilliant Blue R 250.

determine whether it was tissue PA or urokinase. The PA activity in conditioned medium from human melanocytes was reduced by 88% in the presence of 50 μg/ml of anti-tissue PA IgG (Fig. 2). Dose-dependent inhibition was also observed. Anti-urokinase IgG did not affect the PA activity in conditioned medium.

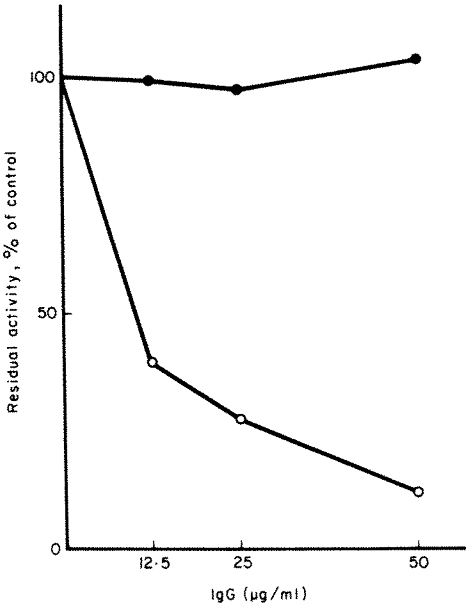

FIGURE 2. Effect on melanocyte PA activity of anti-urokinase IgG (●——●) and anti-tissue PA IgG (○——○).

## DISCUSSION

Recently, PAs secreted by a wide variety of cells have been classified into two types, urokinase and tissue PA, which can be distinguished both immunologically and genetically (Günzler *et al.*, 1982; Pennica *et al.*, 1983).

Stimulation of PA production by TPA in a variety of cultured cell types has been reported (Wigler & Weinstein, 1976; Vassalli, Hamilton & Reich, 1977). It is possible that PA production by human melanocytes in our culture system might have been stimulated by TPA. However, the stimulating effect is not universal. Brown (1982) reported that while TPA increased synthesis of DNA in cultured mouse epidermal cells, the PA activity was not affected. We observed no clear stimulation of PA production by TPA in cultured human epidermal cells (Hashimoto *et al.*, 1983b).

Our results show that cultured human melanocytes secrete tissue PA of $M_r$ 72 000.

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**Pharmacology and Treatment**

# Treatment of chronic discoid lupus erythematosus with an oral gold compound (auranofin)

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## SUMMARY

Twenty-three patients with severe longstanding discoid lupus erythematosus, unresponsive to conventional treatments, were treated with oral gold in a multicentre open study. Nineteen patients showed clinical improvement and in four of these there was complete resolution of lesions. Adverse reactions were generally mild and self limiting.

Systemically administered gold salts were commonly used for the treatment of discoid lupus erythematosus (DLE) from their introduction in the 1920s until the advent of the synthetic antimalarials in the 1950s. There were many reports of the efficacy of gold in large numbers of patients (Wright, 1936; Bechet, 1942). Since chloroquine and its derivatives, potent topical steroids and cosmetically acceptable sunscreens have been available, many patients with DLE can be treated effectively with less risk of the severe toxic side-effects that sometimes resulted from the use of gold salts. Consequently, gold therapy has rarely been considered as an alternative treatment for this condition in recent years. However, there remains a group of patients with disfiguring disease who do not respond to conventional therapy. The introduction of a new orally-administered gold preparation, auranofin, offered the possibility of a known effective treatment, gold, with the convenience of an oral medication and a lessened risk of adverse reactions.

Auranofin [(1-thio-β-D-glucopyranosato) (triethylphosphine) gold 2,3,4,6, tetra acetate] was developed for the treatment of rheumatoid arthritis (Wright, 1984). In contrast to gold sodium thiomalate, auranofin gold is absorbed orally, although relatively poorly, is lipid soluble, exhibits protracted blood levels, is excreted mainly in the faeces and exhibits less tissue retention (Mirabelli & Crooke, 1982). Auranofin has anti-arthritic, anti-inflammatory and 'condition-

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This study was presented at the British Association of Dermatologists meeting in London, July 1985.

dependent' immunoregulatory activity. The activated macrophage is the likely target for both the immunoregulatory and anti-inflammatory actions of the drug (Hanna *et al.*, 1982). It is also possible that part of the action of auranofin in relation to skin disorders is a direct effect on the epidermis. Dykes, Jenner and Marks (unpublished data) have shown a dose-dependent inhibition of epidermal cell growth in culture with a decrease in both number and size of cell colonies.

## METHODS

An open study was designed. This was judged to be the most appropriate type of trial, for the following reasons. Only patients with severe disease were to be enrolled, and they were to receive a drug with some potentially serious toxic side-effects. Acknowledged seasonal variation in the DLE disease activity also dictated that the study should be of one year's duration, and it was unacceptable ethically for some patients with severe DLE to remain on placebo therapy for this length of time.

Twenty-three patients (17 female, 6 male) with severe disease, aged between 16 and 75 years (mean age 48 years) were recruited into the study. For the purpose of this study, severe disease was defined as being widespread or markedly disfiguring DLE, present without remission for at least 5 years (mean 15·4 years). None of the patients had responded to topical steroids. All but four patients had been treated previously systemically with antimalarial drugs, two had received oral prednisolone in addition and, in one of these patients, thalidomide had also been tried without benefit. In four cases, antimalarials were contra-indicated or not tolerated. Biopsy confirmation of the diagnosis was obtained at the beginning of the study if it had not been carried out previously. None of the patients had any clinical or biochemical evidence of serious concomitant disease. None had systemic lupus erythematosus as defined by the ARA (1982) criteria.

Other reasons for exclusion from the study were a strong atopic background, a history of sensitivity reactions to heavy metals, and inadequate contraception in women of childbearing age.

All forms of treatment, except sunscreens, were withdrawn for at least one month before commencing auranofin at a dose of 3 mg bd. If there was little or no beneficial response after 3 months, the dose could be increased up to a maximum of 9 mg daily. If after 5 months there was no improvement, a topical fluorinated steroid could be added. The study continued for up to one year.

Patients were seen monthly for 6 months and then 3-monthly until completion of the study. Biochemical tests of renal and hepatic function were carried out at each visit, on peripheral blood samples. In addition, thyroid function, serum uric acid and serum immunoglobulins were measured regularly. Monthly urinalysis was performed and 24 h urinary protein was estimated when indicated. Haematological parameters of full blood count, red cell indices, white cell number and differential count, platelet count, erythrocyte sedimentation rate and anti-nuclear factor were determined. Eight patients also had anti DNA antibodies estimated.

Clinical examination and assessment of disease were carried out using a 0–3 scale for each of erythema, thickness and scaling of lesions. The scoring system was kept as simple as possible in order to minimize differences between the participating centres. Clearly, the data is subjective and can only give a general view of change in disease activity. In several cases, some areas of involved skin showed great improvement or resolution whilst other areas remained unchanged. As far as possible, the same area was assessed at each visit. Scores for each of erythema, thickness and scaling, and a summated score of all three factors before and at the end of treatment were compared using a Wilcoxon matched pairs signed ranks test (a decrease in score indicated improvement). Photographs were taken before treatment and after 5 and 12 months of auranofin therapy.

## RESULTS

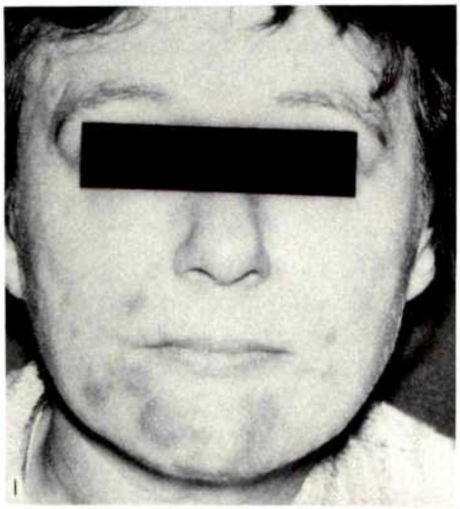
Sixteen of 23 patients completed the study. One patient with severe facial DLE who had previously received oral prednisolone and thalidomide, flared after 2 months of auranofin therapy. Treatment was continued but no improvement occurred and the patient was withdrawn from the study after 9 months. One patient was unable to attend regular follow-up, and withdrew after 4 months. One male patient died of a myocardial infarction after 3 months of treatment. Two patients withdrew from the study with possible adverse clinical reactions and two were withdrawn by the investigators because of abnormal biochemical results.

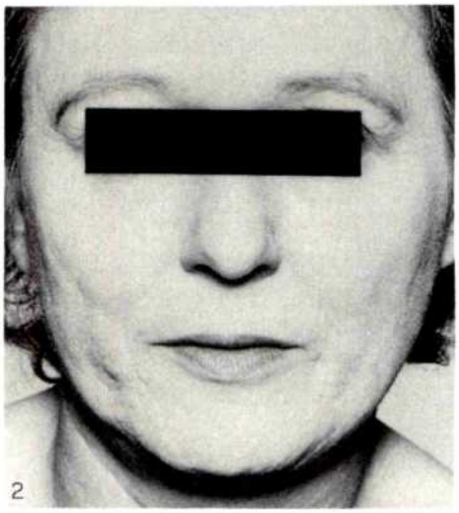
Changes in lesion scores are shown in Table 1. One patient worsened on auranofin, and in three the DLE was unchanged; improvement occurred in 15 and there was resolution of skin lesions in four patients. There was a significant decrease in total scores and in individual scores for erythema, scaling and thickening, both in the whole group of patients, and in the subgroup of those completing the study ($P < 0{\cdot}01$). In general, patients with papular 'rosacea-like' DLE did best, with complete resolution of the lesions and no residual scarring. The woman illustrated in Figure 1, with papular DLE of 10 years duration, had found topical steroids unhelpful and had been unable to tolerate hydroxychloroquine. Marked improvement occurred within 4 months of commencing auranofin and lesions had cleared by 9 months (Fig. 2). Therapy was stopped after one year and the patient has so far remained free of disease for 9 months.

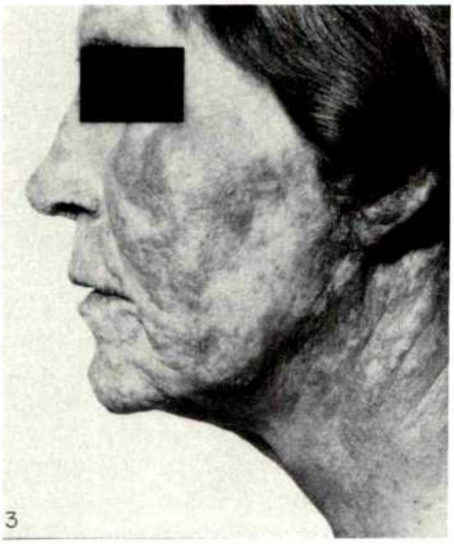
Thicker, plaque-type DLE of the face and upper trunk also responded well, with reduction in active disease characterized by improvement in colour and texture of lesional skin with a reduction in soreness and itching. Residual atrophic scarring was seen in these cases as it is with other forms of treatment. Grossly disfiguring facial DLE had forced the woman shown in Figure 3 to become a virtual recluse. Conventional therapies and systemic steroids had been unhelpful. Early in the course of auranofin a change in skin texture could be detected with softening of the warty areas. Considerable flattening of lesions, with marked reduction in erythema and pigmentation occurred during the year of treatment (Fig. 4). Clearly, in patients with such disfiguring and intractable disease, this type of result represents a major improvement

TABLE 1. Number of patients showing reduction in lesion scores after treatment with auranofin

| | Reduction in score | | | | |
|---|---|---|---|---|---|
| | 3<br>Very marked improvement | 2<br>Marked improvement | 1<br>Improvement | 0<br>No change | −1<br>Deterioration |
| Erythema | 1 | 7 | 10 | 4 | 1 |
| Scaling | 0 | 7 | 8 | 4 | 4 |
| Thickness | 0 | 3 | 12 | 8 | 0 |







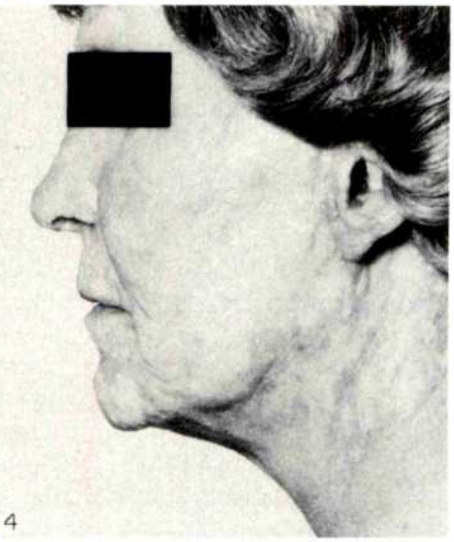


FIGURE 1. Non-scarring facial DLE of 10 years duration before treatment.

FIGURE 2. Nine months after completing auranofin the disease remains in remission.

FIGURE 3. Severe scarring DLE unresponsive to antimalarials and systemic steroids.

FIGURE 4. Marked reduction in erythema, pigmentation and thickness of lesions after one year of auranofin treatment.

in the quality of life. Warty nodules and plaques of DLE on the limbs responded least well, even with the addition of a topical fluorinated steroid.

Attempts to monitor disease activity by haematological parameters were unsuccessful. Antinuclear antibodies (ANA) were elevated in one patient throughout the treatment period. A further four patients showed intermittently elevated titres of ANA which did not correlate with clinical assessment of disease activity. Anti DNA antibodies were measured in eight patients but again, titres varied and did not correlate with disease activity or with ANA levels in parallel samples. There was no appreciable change in the erythrocyte sedimentation rate during treatment.

Possible adverse reactions were documented at each visit. Specific enquiry was made regarding the occurrence of conjunctivitis, diarrhoea, pruritus, skin rash and stomatitis. The patient was also asked to describe any other abnormal events. Grastro-intestinal effects were most obvious. One patient withdrew from the study with nausea and vomiting in the first month of treatment. Loose diarrhoeal stools were common (10 patients) but this was usually mild and settled spontaneously over a few weeks without stopping treatment. Three patients experienced pruritus within lesions of DLE. This was mild in two cases but severe in one woman who withdrew from the study. Two patients developed transient rashes—one a macular erythema on the trunk which was asymptomatic and settled spontaneously without stopping auranofin; the other patient complained of an erythematous facial rash which faded before being seen by the investigator. Two patients developed a sore mouth and tongue; one of these experienced mild aphthous ulceration periodically throughout the study. No haematological abnormalities were noted; in particular, no evidence of neutropenia or thrombocytopenia was seen. Four patients showed a modestly elevated alkaline phosphatase (AP) before treatment. In one of these patients the AP rose to a maximum of 166 IU/l (normal values 30–95 IU/l) and she was withdrawn from the study. No other abnormalitiy in hepatic function was found and no underlying disease detected. One patient developed proteinuria which peaked at 0·8 mg/24 h. She was withdrawn from the study and the proteinuria settled over 4 months. No other abnormality of renal function was detected during the study.

## DISCUSSION

This study has shown auranofin to be a useful additional therapy in the treatment of severe chronic discoid lupus erythematosus. It has made life much more tolerable for several of our more severely afflicted patients. At present, the drug does not have a licence for use in DLE but is available on application to the pharmaceutical company* who developed and manufacture it for use by hospital specialists in carefully selected, named patients who can be closely monitored.

Clearly, the potential toxicity of gold compounds remains a factor limiting their use at present. The adverse reactions documented in this study have also been recorded in patients receiving auranofin for the treatment of rheumatoid arthritis. In a study of 3082 patients receiving auranofin for up to 4 years for rheumatoid arthritis, Heuer and Morris (1982) showed that 11·2% of patients were withdrawn from studies because of adverse effects. This compared with a withdrawal rate of 25% in 465 patients receiving injectable gold for periods of approximately 6 months. Adverse effects were very common in both groups, occurring in 71·3% of patients treated with auranofin. Most adverse reactions were mild, of short duration and were tolerated as treatment continued, or resolved on reducing the dose. Loose stools and diarrhoea were the commonest problems occurring in almost 40% of patients, although only 3% of patients stopped auranofin treatment because of this reaction. Other common reactions were rash (18%), gastro-intestinal disturbance (17%), pruritus (13·4%) and conjunctivitis and oral stomatitis (10% each). Again, only small numbers withdrew from treatment because of these

*Smith, Kline & French, Welwyn Garden City, Hertfordshire, U.K problems. Thrombocytopenia and leucopenia were each seen in under 2·5% of patients but only 0·4% were withdrawn with persisting proteinuria. Despite the relatively low incidence of these problems, this data reinforces that all patients receiving auranofin must be monitored closely. No case of drug-related myocardial infarction has been demonstrated, and we do not regard the death of one subject from this disorder as being related in any way to the treatment.

In view of the small number of subjects who have received the drug for DLE, it is not possible to speculate whether the incidence of adverse reactions will be similar to that seen in rheumatoid arthritis patients.

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# Itraconazole therapy in pityriasis versicolor

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## SUMMARY

The efficacy of itraconazole was assessed in an open trial in 30 patients with disseminated lesions of pityriasis versicolor confirmed by direct microscopy. The patients were allocated randomly to one of two treatment regimens, 200 mg once daily for 5 days or 100 mg once daily for 10 days. On assessment 3 weeks after the end of the treatment, 25 patients were healed, two patients had mild residual lesions, two had considerable residual lesions and one patient had relapsed. One patient reported dyspepsia and one patient reported stomach ache. One patient had asymptomatic elevation of serum transaminase (GOT and GPT) but this had returned to normal 3 weeks after the end of therapy.

Pityriasis versicolor is the most common superficial mycotic disease world-wide and is caused by *Malassezia furfur* (Roberts, 1969a). *Pityrosporum orbiculare* and *P. ovale*, are both lipophilic members of the normal human cutaneous flora, and are probably identical (Faergemann & Fredriksson, 1982). *M. furfur* is thought to be the filamentous form of *P. orbiculare* (*P. ovale*) and this is now considered the correct name under which *P. ovale* and *P. orbiculare* are combined (Salkin & Gordon, 1977). *Pityrosporum sp.* develops from the saprophytic yeast form to the pathogenic mycelial form under the influence of several factors, including seborrheic dermatitis (Faergemann & Fredriksson, 1979), hyperhidrosis (Burke, 1961; Roberts, 1969a, b), genetic factors (Burke, 1961; Faergemann & Fredriksson, 1982; Roberts, 1969a, b) systemic corticosteroid therapy, malnutrition and immunosuppressive treatment (Burke, 1961; Roberts, 1969b). Sonhle and Collins-Lech (1978) found a defect in the production of lymphokines in patients with pityriasis versicolor.

Spontaneous improvement is uncommon in pityriasis versicolor, and left untreated it tends to persist for many years. Although a few cases clear spontaneously in cool weather, the majority of patients require therapy. Treatment of pityriasis versicolor is simple, but relapse is common, as well as depigmentation of infection sites. Pityriasis versicolor used to be treated with a wide range of topical compounds (Ive, 1973; Hay, 1981), including selenium sulphide, thiosulphate,

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keratolytic agents, propylene glycol and topical antifungals. Patients can experience difficulty in applying topical preparations, especially on large areas of the body and often the treatment is inadequate. In many instances, the choice of treatment is made on the basis of simplificity of usage and patient acceptability.

Ketoconazole was the first oral antifungal effective in the treatment of pityriasis versicolor (Borelli, 1980; Palacio-Hernanz and Merino, 1981) and clinical trials have confirmed that treatment periods of between 5 and 28 days give satisfactory results (Jolliffe & Ngai, 1981; Urcuyo & Zaias, 1982; Savin, 1984; Meisel, 1983; Hay & Midgeley, 1984). The majority of patients find this treatment acceptable; its main disadvantage is the risk, albeit low, of liver toxicity and the possibility of endocrine affects (Hanifin, 1983; Janssen & Symoens, 1983; Schuermeyer & Nieschlag, 1982).

Itraconazole is a triazole derivative which has been shown to be highly active *in vitro* against a wide range of fungi including *Pityrosporum sp.* (van Cutsem, van Gerven & Janssen, 1985; Faergemann, 1984). Animal studies have indicated that this drug, when administered orally is 5–10 times more potent than ketoconazole (van Cutsem, 1983; van Cutsem *et al.*, 1983), and these animal data have been confirmed in clinical pilot studies (Janssen Pharmaceutica, unpublished data). Toxicity studies have revealed no significant toxicity even at high doses in dogs and rats (Janssen Pharmaceutica, unpublished data), nor any effect on steroidogenesis in animals and human volunteers (R. de Coster, personal communication). Teratogenicity studies were positive in rats receiving high doses of itraconazole (40 mg/kg and above), indicating that this drug should not be used during pregnancy (Janssen Pharmaceutica, unpublished data).

We report here the results of a radomized open study of two treatment regimens with itraconazole in patients with extensive pityriasis versicolor.

## METHODS

### *Patient selection*

Thirty patients with disseminated skin lesions consistent with pityriasis versicolor, who were seen in our department over a 2-month period, were included in the trial. The criteria for inclusion were skin lesions typical of this condition (erythema, scaling, hyper- and hypopigmentation), fluorescence in Wood's light and positive microscopic diagnosis with potassium hydroxide (KOH).

All patients were assessed prior to treatment with a detailed history and clinical examination. Four parameters of clinical disease activity (itching, scaling, erythema and hypo- or hyperpigmentation) were assessed and scored as: 0 = none; 1 = mild; 2 = moderate; 3 = severe. Patients in any of the following categories were excluded: less than 17 years of age; presence of systemic mycotic disease; women who were pregnant or breast-feeding, or those who were not on reliable contraceptives; systemic or topical antimycotic treatment within one month prior to the trial; serious concurrent disease that might prevent completion of therapy. Written consent was obtained from all patients.

At the initial visit a blood sample was taken for haematology (packed cell volume, haemoglobin, red cell count, leukocyte count with differential count, and platelets) and biochemistry (glucose, creatinine, alkaline phosphatase, $\gamma$GT, bilirubin, SGOT, SGPT, LDH, cholesterol and triglycerides).

### *Medication*

Thirty patients were allocated randomly, on an open basis, to either regimen I (15 patients), which consisted of 200 mg o.d. of itraconazole taken at breakfast for 5 consecutive days, or regimen II (15 patients), which consisted of 100 mg o.d. of itraconazole for 10 consecutive days. No other medication was allowed during the study.

### *Follow-up*

The patients were asked to return one day after the end of therapy and again 3 weeks later. At these visits, the patients were assessed in the same way as at the pre-treatment visit. All the clinical evaluations were carried out by the same person.

At the end of the trial, the patient's condition was rated as healed, mild residual lesions, considerable residual lesions, unchanged, deteriorated and unevaluable. Patients with complete healing or residual lesions were considered *responders*. Adverse effects were recorded and rated according to severity. Haematological and biochemical evaluations were repeated.

### *Mycological examination*

At the first visit, scrapings were taken from the lesions for mycological assessment: light microscopy with potassium hydroxide, culture of *Pityrisporum sp.*, electron microscopy and study of MIC of intraconazole. The culture medium was Sabouraud dextrose agar (Difco) with 0·5 g/l cycloheximide and 0·05 g/l of cloramphenicol, and olive oil overlay. Tubes with scrapings were incubated at 37°C and maintained on a slope to keep the agar slant covered with oil. In all patients *Pityrosporum sp.* was grown before starting treatment. Scales for electron microscopy were fixed in glutaraldehyde and processed routinely for ultrastructural examination. Before treatment, scanning electron microscopy showed normal size *Pityrisporum* yeasts as well as short hyphae in all patients. Transmission electron microscopy revealed yeasts with thick cell walls and short mycelia with a normal intracellular structure. In some patients a small percentage of dead yeasts was observed before therapy.

### *Sensitivity testing*

When growth was visible on the isolation medium, single colonies were sub-cultured onto Dixon agar. For sensitivity testing, cultures in 5 ml Dixon broth were incubated at 37°C for 4 days and dilutions made in Dixon broth to give final concentrations in the test medium of between 25 000 and 30 000 colony forming units per ml. Stock solutions (2 mg/ml) of miconazole and ketoconazole were prepared in 50° ethanol and of itraconazole in DMSO. Drugs were added to the test medium to give final concentrations from 100 $\mu$g/ml to 0·001 $\mu$g/ml. Controls with and without the solvents were included and all experiments were performed in duplicate.

Tubes were observed for visible growth after 7 and 14 days incubation at 37°C. After 14 days, the tubes without macroscopically visible growth were subcultured to distinguish fungistatic from fungicidal activity.

## RESULTS

The patients' characteristics and history are shown in Table 1. In seven patients in each group pityriasis versicolor was present for the first time and these patients had not had previous treatment. The mean duration of this episode was 24 months in Group I and 64 months in Group II.

Eight patients in each group had recurrent pityriasis versicolor and they had had different treatments with a variety of topical compounds (selenium sulphide, zince pyrithione, propylene glycol and topical imidazoles). The majority of these patients had had several relapses.

TABLE 1. Clinical details of patients in the trial

| | Group I (15 patients) | Group II (15 patients) |
|---|---|---|
| Sex: male | 5 | 11 |
| female | 10 | 4 |
| Age (years): mean (range) | 30·1 (18–71) | 26·2 (17–54) |
| Weight (kg): mean (range) | 61·6 (44–82) | 67·8 (54–90) |
| 1st episode of pityriasis versicolor: no. of patients | 7 | 7 |
| Duration of first episode (months) mean (range) | 24 (1–96) | 63·5 (1–360) |
| Recurrent pityriasis versicolor: no. of patients | 8 | 8 |
| Recurrent pityriasis vesicolor: duration (months) mean (range) | 57 (6–72) | 36 (12–120) |
| Duration of last episode of recurrent pityriasis versicolor (months) mean (range) | 2 (1–7) | 2·5 (2–7) |

All patients in both groups had numular lesions but 12 also had confluent lesions. The lesions were located on the trunk, neck and upper arms. Three patients also had lesions on their thighs, two on the groin and three on the face. The lesions were erythematous or hypopigmented, except for 13 patients who had hyperpigmented lesions. Thirteen patients noted mild to moderate itching. All patients had scaling (10 mild, 14 moderate and 6 severe).

### *Assessment one day after the end of therapy*

Table 2 shows the results of the treatment one day after the end of therapy. Itching and erythema had disappeared in both groups of patients. Seven patients in Group I and 10 patients in Group II had mild scaling. Six patients in Group I and one patient in Group II had hyperpigmentation and four patients in Group II had both hyper- and hypopigmentation. Nine patients in Group I and 10 patients in Group II had moderate hypopigmentation.

TABLE 2. Assessment one day after end of therapy

| Parameter | No. of patients (mean clinical score) Group I (15 patients) | No. of patients (mean clinical score) Group II (15 patients) |
|---|---|---|
| Pruritus | 0 (0) | 0 (0) |
| Scaling | 7 (1) and<br>8 (0) | 10 (1) and<br>5 (0) |
| Erythema | 0 (0) | 0 (0) |
| Hyperpigmentation | 6 (1) | 5 (1)* |
| Hypopigmentation | 9 (2) | 14 (2)** |
| KOH light microscopy + ve | 15 | 13 |
| Wood's light + ve | 14 | 13 |
| Culture of *Pityrosporum sp.* + ve | 3 | 6 |

Clinical scores: 0 = none, 1 = mild, 2 = moderate, 3 = severe.

* One patient had hyperpigmentation; four patients had hyper- and hypopigmentation.

** Ten patients had hypopigmentation; four patients had hyper- and hypopigmentation.

Light microscopy with KOH was positive in all patients in Group I and 13 patients in Group II. In some patients who still had mild scaling after treatment, the yeast and the mycelial form could be observed. Transmission electron microscopy showed that the intracellular structure of the cells had changed.

Electron microscopy showed a much smaller number of yeast cells and mycelium at the end of treatment, and the proportion of cells with a completely necrotic interior was markedly increased. However, cells with a completely degenerate cytoplasm, often loaded with lipid globules, retained an intact cell wall. This was confirmed by scanning electron microscopy, which showed that the surface of the cells remained as smooth as those in the pre-treatment sample. Thus, itraconazole appears to have a 'mummifying' effect on *P. ovale.*

Wood's light was positive in 14 patients in Group I and in 13 patients in Group II. *Pityrosporum sp.* was grown from three patients in Group I and from six patients in Group II.

The antifungal activity of miconazole, ketoconazole and itraconazole against seven strains of *Pityrosporum sp.* isolated from the patients is given in Table 3. Miconazole was fungicidal at 10 μg/ml in six strains and at 100 μg/ml for one. 1 μg/ml gave more than 90% growth inhibition and partial growth inhibition was obtained at lower concentrations. Ketoconazole was fungicidal at a concentration of 1 μg/ml; itraconazole, however, was fungicidal for six out of seven strains at 0·1 μg/ml and in one at 1 μg/ml. Fungistatic activity was observed at lower concentrations of all three drugs. None of the strains isolated after therapy was stopped was shown to have developed *in vitro* resistance to itraconazole.

TABLE 3. Antifungal activity of azoles against *Pityrosporum sp.* in Dixon broth (broth decimal test)

| | Concentration μg/ml | | | | | |
|---|---|---|---|---|---|---|
| | Miconazole | | Ketoconazole | | Itraconazole | |
| *P. ovale* strains | fungicidal | markedly fungistatic* | fungicidal | markedly fungistatic | fungicidal | markedly fungistatic |
| 1. B 40689 (7.2826 no. 2) | 10 | 1 | 1 | 0·1 | 0·1 | — |
| 2. B 40690 (8.1496 no. 2) | 10 | 1 | 1 | — | 0·1 | — |
| 3. B 40691 (8.2946 no. 9) | 10 | 1 | 1 | 0·1 | 0·1 | — |
| 4. B 40830 (9.1855 no. 13) | 10 | 1 | 1 | — | 0·1 | — |
| 5. B 40831 (9.2013 no. 17) | 100 | 1 | 1 | — | 1 | 0·1 |
| 6. B 40832 (9.0179 no. 18) | 10 | 1 | 1 | — | 0·1 | — |
| 7. B 40836 (9.0763 no. 22) | 10 | 1 | 1 | — | 0·1 | — |

* Marked fungistatic activity: more than 90% versus controls.
— = endpoint not observed.

### *Assessment 3 weeks after therapy*

Table 4 shows the results of the global assessment of treatment 3 weeks after finishing therapy. Fourteen patients (93%) in Group I had responded to treatment; 13 patients were completely healed and one had mild residual lesions. One patient had considerable residual lesions. Thirteen patients (87%) in Group II had responded to the therapy; 12 patients were completely healed and one had mild residual lesions; one patient had considerable residual lesions and one patient had deteriorated.

*Pityrosporum sp.* was cultured from four patients in Group I (two patients who were healed, one with mild residual lesions and one with considerable residual lesions), and from six patients in Group II (three patients who were healed, one who had mild residual lesions, one who had considerable residual lesions and one who had deteriorated). In Group I, KOH and Wood's light were positive in two patients (one patient with considerable residual lesions and the other with mild residual lesions).

At the second evaluation, only material from patients who were not cured was studied by electron microscopy. In these few samples both the yeast form and the short mycelial form were detected; transmission electron microscopy showed that these few organisms had a normal intracellular structure.

In Group II, KOH and Wood's light were positive in three patients (one with considerable residual lesions one who had deteriorated and one with mild residual lesions). Depigmentation remained in seven patients in Group I and 10 patients in Group II (one of whom had

TABLE 4. Assessment 3 weeks after the end of therapy

| Parameter | | No. of patients | | |
|---|---|---|---|---|
| | | Group I | Group II | Total |
| Responders: | healed | 13 | 12 | 25 |
| | mild residual lesions | 1 | 1[1] | 2 |
| | Total (%) | 14 (93) | 13 (87) | 27 (90) |
| Considerable residual lesions | | 1 | 1[2] | 2 |
| Unchanged | | 0 | 0 | 0 |
| Deteriorated or relapsed | | 0 | 1 | 1 |
| Positive culture of *Pytırosporum sp.* | | 4[3] | 6[4] | 10 |
| Positive KOH microscopy | | 3[5] | 3[6] | 6 |
| No residual lesions | | 5 | 3 | 8 |
| Residual hypopigmentation only | | 7 | 9 | 16 |
| Residual hyperpigmentation only | | 1 | 0 | 1 |

Responders = patients with complete healing or mild residual lesions.

[1] With hyperpigmentation.

[2] With hypopigmentation.

[3] Two patients were healed, one had mild residual lesions and one had considerable residual lesions.

[4] Three patients were healed, one had mild residual lesions, one had considerable residual lesions and one had deteriorated.

[5] One patient was healed, one had mild residual lesions and one had considerable residual lesions.

[6] One patient had mild residual lesions, one had relapsed and one had considerable residual lesions.

considerable residual lesions). Eight patients who sunbathed after treatment were evenly tanned and had no residual lesions. One patient in Group I had mild hyperpigmentation without scaling on a restricted area on the side of the neck, but was mycologically cured (Wood's light, KOH direct microscopy and culture for *Pityrosporum sp.* were negative). The patient in Group II who had mild hyperpigmentation and scaling was evaluated in the final assessment as having mild residual lesions, and KOH, Wood's light and culture for *Pityrosporum sp.* were positive.

### *Three-month follow-up*

All patients were questioned by telephone 3 months after the end of treatment. All patients who had cleared 3 weeks after the end of therapy, were still clear 3 months later. In most patients repigmentation had occurred.

### *Acceptability*

All the patients found the treatment simple, easy and acceptable. Compliance was very good, probably because the medication was given in a single daily dose and for a short period of time.

### *Adverse reactions*

Two adverse reactions were reported in Group I. One patient experienced mild dyspeptic symptoms with each dose, that lasted for 2–3 h after ingestion of the drug. Another patient reported mild stomach ache for 1 h following each dose. Both patients were able to complete the treatment schedule.

### *Haematology and biochemistry*

One patient in Group II presented significantly abnormal GOT and GPT values at the end of the treatment (GOT 121 IU/l, GPT 131 IU/l, normal range 0–45 IU/l. This increase was asymptomatic and had completely normalized by the 3-week follow-up. No other significant haematological or biochemical abnormalities were observed.

## DISCUSSION

At the assessment one day after the end of therapy, itching and erythema had disappeared in all patients in both groups. However, mild scaling persisted in seven patients in Group I and in 10 patients in Group II. Almost all patients had a positive KOH test. Wood's light was also still positive in these patients, although the intensity of the fluorescence was reduced when compared with that prior to treatment. Electron microscopy showed that in many of the patients who still had some scaling with a positive KOH test, fungal elements had been altered or destroyed, and it was observed that cells could become 'mummified' after treatment, with dead cells retaining an intact surface structure.

Comparing the results immediately after the end of treatment and 3 weeks later, it is evident that the final clinical and mycological evaluation of a treatment of pityriasis versicolor should be performed at least 3 weeks after the end of treatment. The KOH test and Wood's light examination are not relevant immediately after the end of the therapy.

*Pityrosporum orbiculare* (*P. ovale*) is considered to be the aetiological agent of tinea versicolor (Roberts & Mackenzie, 1979; Faergemann & Fredriksson 1982; Salkin & Gordon, 1977). This organism was cultured from all patients prior to therapy, from nine patients at the end of treatment and from 10 patients 3 weeks later. At this time, five of the patients with a positive culture were assessed as healed and no fungi were seen microscopically, which suggested that they were carrying the yeast as a saprophyte on their skin. Faergemann and Djärv (1982) reported a similar finding on the last day of treatment in 10 healed patients with pityriasis versicolor, who had been treated with 200 mg oral ketoconazole o.d. for 3 weeks. In 20 patients in our study, culture of *Pityrisporum sp.* 3 weeks after the end of treatment was negative; these findings demonstrate the prolonged pityrosporicidal effect of itraconazole even when treatment is ended. These findings make the evaluation of a treatment of pityriasis versicolor rather difficult. Cultures give no definitive answer since *Pityrosporum sp.* lives on the skin of many people in a saprophytic form. Microscopy and Wood's lamp are not valid criteria when the assessment is done immediately after treatment with an orally active antifungal, since dead fungal elements remain on the skin. Valid assessment of the efficacy of an oral treatment appears to be possible only a minimum of 3 weeks after end of therapy. At this time, both microscopy and clinical aspects should be evaluated. Hypopigmentation cannot be regarded as a primary symptom of the infection since the melanocytes need some time to recover even after disappearance of the fungus from the skin.

Clinically mild side-effects (mild dyspeptic symptoms and stomach ache) only appeared in two patients in Group I and were probably related to the higher daily dose of the drug.

Sensitivity to the three azoles of the seven *Pityriosporum* strains tested was comparable to those obtained in previous experiments (Faergemann, 1984; Faergemann & Djärv, 1982; Faergemann 1979; Faergemann & Bernander, 1979). Miconazole possesses high activity *in vitro* against *Pityrosporum sp.* and *in vivo* in pityriasis versicolor after topical application (Faergemann & Djärv, 1982; Faergemann & Bernander, 1979). The most important problems with topical imidazole treatment are the untreated lesions and the presence of the yeast as a saprophyte in the follicles. Ketoconazole is more active *in vitro*, and is absorbed systemically after oral intake and distributed as the active substance to the areas involved (van Cutsem, 1983). The *in vitro* activity of itraconazole is about 100 and about 10 times more potent than those of miconazole and ketoconazole respectively (Janssen Pharmaceutica, 1984; Faergemann & Djärv, 1982). Most important is its superiority over ketoconazole, suggesting possible greater activity *in vivo*.

Asymptomatic increases of liver enzymes have been observed during the first days of treatment with all drugs that are extensively metabolized in the liver (Petersen, Alling & Kirkpatrick, 1980; Drouhet & Dupont, 1983). Although our finding with itraconazole in one patient is not necessarily relevant to the general population, it remains necessary to monitor liver function in patients who receive long-term therapy with this drug.

In our study, the response to therapy did not seem to be influenced significantly by the existence of previous episodes of pityriasis versicolor or by the duration of the episode being treated. The introduction of ketoconazole made oral treatment of pityriasis versicolor possible (Borelli, 1980), an important advance since non-compliance with topical therapy is common, and oral drugs, particularly given in a single dose or a short course, may produce better compliance. Itraconazole seems to be an effective agent for the treatment of pityriasis versicolor; however, optimum treatment schedules and more clinical experience with longer follow-up periods are required to determine the efficacy and safety of the drug. Itraconazole should also be compared under double-blind conditions with alternative agents.

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# Oral spironolactone: an effective treatment for acne vulgaris in women

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## SUMMARY

The effect of oral spironolactone (200 mg daily) on acne vulgaris has been studied in 21 women in a randomized, placebo-controlled, double-blind crossover study using 3 month treatment periods. Compared with placebo, spironolactone produced significant improvement as assessed by subjective benefit ($P < 0{\cdot}001$), number of inflamed lesions ($P < 0{\cdot}001$) and by an independently evaluated photographic method ($P < 0{\cdot}02$). There was a fall in sex hormone binding globulin but no significant changes in plasma testosterone and derived free testosterone. Initial plasma androgen levels were no higher in responders than in non-responders, nor did oral contraceptive use appear to affect clinical response. Spironolactone is a useful alternative therapy for women with acne vulgaris.

Spironolactone is an aldosterone antagonist and an anti-androgen. Its anti-androgenic properties have been shown to be beneficial in the treatment of female hirsutism (Ober & Hennessey, 1978) and in acne vulgaris (Boisselle & Tremblay, 1978). In a recent study at this hospital in patients with acne, spironolactone was shown to reduce sebum excretion rates and the clinical effect was found to be dose-dependent (Goodfellow *et al.*, 1984). We have evaluated further the usefulness of spironolactone (200 mg daily) for female patients with acne, and monitored its effect on their androgen status.

## METHODS

Twenty-nine women with moderate to severe acne entered the trial. No antibiotics were administered during the month that preceded the trial and no topical treatment was allowed during the study. Six patients continued previously prescribed oral contraceptives during the study period. The trial was a double-blind, placebo-controlled crossover study, patients being allocated randomly by a sealed envelope method to commence treatment with either 200 mg daily of spironolactone (Group A) or placebo (Group B). After the first 3 months, patients were

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given one month of placebo and crossed over to a further 3 months of either placebo or spironolactone respectively.

Response to treatment was assessed by objective and subjective methods and all patients were seen by the same observer (M.F.M). At each interview patients were asked to grade their acne on a five-point scale (much worse, worse, unchanged, better, much better). At each visit only the inflammed lesions were counted—papules, pustules and nodules. Improvement was defined as a greater than 50% reduction in the number of lesions and worsening as a greater than 50% increase. Monochrome photographs were also taken at each visit and then assessed double-blind by the three co-authors who were not involved in patient supervision. The subjective and the objective responses were recorded as worse, unchanged or improved.

At each visit blood was taken for assay of plasma testosterone and sex hormone binding globulin (SHBG). Testosterone was assayed by the method of Collins *et al.* (1972), SHBG by the method of Iqbal and Johnson (1977) and derived free testosterone (DFT) calculated as described by Anderson, (1976). The results are presented as means ± S.E.M.

The statistical tests used were the paired $t$-test, $\chi^2$ with Yates's correction, or the Mann-Whitney U test.

## RESULTS

Of the original 29 patients, 21 completed the trial. Two patients withdrew due to adverse side-effects and six failed to return after the initial interview.

### *Clinical changes*

Figure 1 shows the changes in mean lesion count with time for the two groups. By chance,

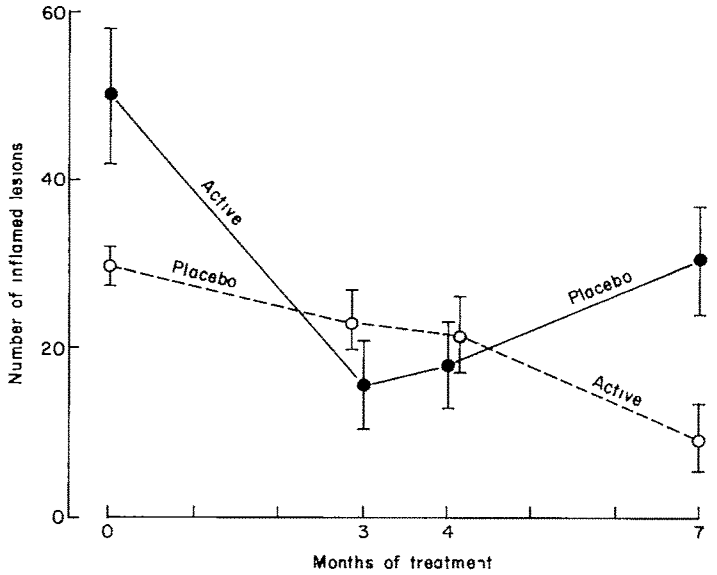

FIGURE 1. Numbers of inflamed lesions in relation to treatment with spironolactone and placebo in Group A ●——●, and Group B ○– – –○. Points are means ± SEM.

patients who began with spironolactone had a higher initial lesion count. Taking the pooled data from Groups A and B, the mean lesion count fell from 37·8 ± 5·8 to 12·9 ± 3·3 on spironolactone ($P < 0{\cdot}001$, paired *t*-test) and was virtually unaltered on placebo (23·5 ± 3·2 to 24·7 ± 3·9; $P > 0{\cdot}1$).

Figure 2 shows the proportion of patients who were worse, improved or unchanged on spironolactone or placebo. The beneficial effect of spironolactone was apparent as assessed by all

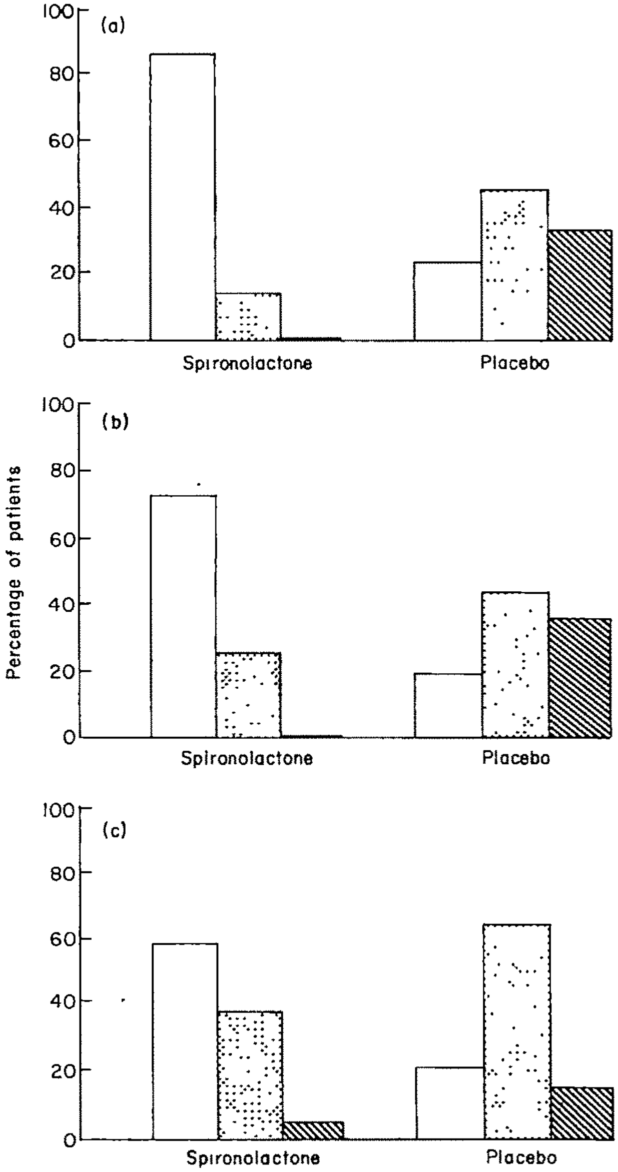

FIGURE 2. Proportions of patients who were improved (□), unchanged (▨) and worse (■) on spironolactone and on placebo, evaluated by three separate methods. (The objective assessment was based on inflamed lesion counts.) (a) Subjective (patient; $n = 21$); (b) objective (lesion count; $n = 20$); (c) photographic ($n = 19$).

three methods. Eighteen patients (86%) noted improvement on spironolactone whilst only five (24%) did so on placebo ($P<0{\cdot}001$, $\chi^2$ test). On the basis of lesion counts, 15 patients (75%) improved on spironolactone and four patients (20%) improved on placebo ($P<0{\cdot}001$, $\chi^2$ test). By the photographic method 11 patients (58%) were better on spironolactone as compared with only four (21%) on placebo ($P<0{\cdot}02$, $\chi^2$ test). There was no effect of oral contraceptives on clinical response.

### *Adverse effects*

The most frequently reported side-effect was menstrual irregularity which occurred in 11 of the 15 patients who were not on the contraceptive pill. None of those affected discontinued treatment, and menses returned to normal within one month of stopping therapy. Nausea was reported by three patients and dizziness by one. The two patients who withdrew from the trial whilst on spironolactone complained of nausea. Nine patients noted that their skin and hair was less greasy and two developed some breast enlargement. These side-effects were considered beneficial.

### *Endocrine results*

In response to spironolactone SHBG fell from a mean of 48 ± 4·6 to 39 ± 3·4 nmols DHT bound/l ($P<0{\cdot}001$, paired *t*-test); mean plasma testosterone did not change (2·0 ± 0·1 and 2·0 ± 0·3 nmols/l; $P>0{\cdot}1$); DFT also did not change significantly (pre-treatment 27·8 ± 2·0; post-treatment 29·6 ± 4·8 pmols/l; $P>0{\cdot}1$), and no significant changes were seen during treatment with placebo. There was no correlation between clinical response and initial androgen levels. The initial DFT for those patients who improved was 27·6 ± 2·3 pmols/l compared with 28·0 ± 3·7 pmols/l for those who failed to respond ($P>0{\cdot}1$). Patients receiving oral contraceptives had lower initial mean testosterone levels than those who were not (1·6 ± 0·1 compared with 2·1 ± 0·1 nmol/l; $P<0{\cdot}05$; Mann Whitney test), higher SHBG levels (83·0 ± 19·9 compared with 48·5 ± 4·6 nmol DFT bound/l; $P>0{\cdot}1$), and lower DFT levels (17·7 ± 3·0 compared with 27·8 ± 1·9 pmol/l; $P<0{\cdot}02$).

## DISCUSSION

The ways in which spironolactone exerts its anti-androgenic effects are not fully understood. It appears to inhibit testosterone biosynthesis by blocking cytochrome P450 enzymes in the adrenal gland and testis (Menard, Stripp & Gillette, 1974) and may also affect ovarian androgen synthesis. Spironolactone also acts peripherally by inhibiting the binding of diyhdrotestosterone (DHT) to nuclear and cytosolic receptors in target tissues such as the skin and prostate (Corvol *et al.*, 1975). Sebaceous gland activity is regulated by androgens (Strauss & Pochi, 1974) and is affected consequently by spironolactone or its chief metabolite potassium canrenoate, blocking the effects of DHT on sebaceous gland androgen receptors.

Although increased sebum production is a major factor in the pathogenesis of acne, androgen status is usually normal. Free testosterone is related to the SHBG level so that changes in SHBG may have an important influence on sebaceous gland activity. However, some women with acne and hirsutism have isolated abnormalities of androgen metabolism (Marynick *et al.*, 1982; Lucky *et al.*, 1983). Spironolactone has been shown to suppress adrenal androgen synthesis (Ricciardi, Del Rio & Imperato, 1974; Walsh & Siiteri, 1975; Loriaux *et al.*, 1976) so that it might be of value in the treatment of patients with acne in whom there are abnormalities of androgen metabolism.

The results of this study are encouraging and in accordance with our previous findings in an open study of dose responsiveness (Goodfellow *et al.*, 1984), where the best subjective and objective effects were seen in patients taking 200 mg spironolactone daily. In the present study, objective improvement was seen in 75% of patients. There were five treatment failures, two of whom were on the contraceptive pill. Spironolactone was tolerated well by most patients, the only major side-effect being menstrual irregularity. The reason for this may be an increase in plasma oestradiol (Shapiro & Evron, 1980), but spironolactone has also been shown to have anti-oestrogenic properties (Lewy *et al.*, 1980) and a progestational action (Shane & Potts, 1978), so that the menstrual dysfunction probably results from complex hormonal interactions and inhibitions. In some patients there was an apparent carry-over effect of spironolactone into the placebo treatment period, and in some the improvement after spironolactone was apparently permanent.

Spironolactone produced a significant fall in SHBG, a finding that has been observed in a previous study in hirsutism at this hospital (Dorrington-Ward *et al.*, 1985). The mechanism remains unexplained, but reduced hepatic SHBG synthesis is one possibility. There was no change in mean plasma testosterone level and although there was a slight rise in DFT as a result of the fall in SHBG, the difference was not significant. Other studies with spironolactone using doses of 150–400 mg daily in hirsutism have shown reductions of up to 50% in plasma testosterone (Ober & Hennessey, 1978; Messina *et al.*, 1983), representing the inhibitory effects of spironolactone on testosterone biosynthesis (Menard *et al.*, 1974), but this effect may not be seen until the sixth month of therapy (Dorrington-Ward *et al.*, 1985). In our study, benefit was noted by many patients within one month of commencement of spironolactone, suggesting that its principal action in acne is via its peripheral anti-androgenic effect. Five of the 21 patients who completed the study had initial DFT levels above the range of 11·4–32·6 pmol/l previously defined for normal females aged 20–35. This is in keeping with our previously published prevalence rate of hyperandrogenism in acne of 20 % (Goodfellow *et al.*, 1984). Nevertheless, there is no evidence that this subgroup was more responsive to spironolactone, plasma androgen levels being no different in responders than non-responders.

Which patients are suitable for spironolactone and how long should treatment last? The side-effect of gynaecomastia encountered in our earlier study (Goodfellow *et al.*, 1984) renders it an unsuitable treatment for men, but we believe that spironolactone should be considered for women whose acne has not responded to antibiotics and for those who have severe and persistent acne with hirsutism, our experience suggests that the latter group is particularly responsive. Side-effects are few, although it is disconcerting that so many patients experienced menstrual disturbance, albeit of minor degree. It is therefore our current practice to give a 6 month course of treatment, beginning with 200 mg and reducing the dose to 100 mg daily after 3 months, provided that a satisfactory response has been obtained. Adverse effects are less likely to occur at lower dosage, although relapse has been observed in some patients on this dose. In view of the success of spironolactone in the treatment of acne and its ability to produce long-lasting remissions in some patients, a study is now planned to compare its efficacy with that of conventional antibiotic therapy.

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# The measurement of the stratum corneum reservoir: a simple method to predict the influence of vehicles on *in vivo* percutaneous absorption

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## SUMMARY

The influence of nine vehicles on *in vivo* percutaneous absorption of benzoic acid has been studied in the hairless rat. Although the vehicles used were simple in composition, the total amount of benzoic acid which penetrated varied by a factor of 50. A weak relationship was found between penetration of benzoic acid and its maximum solubility in the different vehicles. A linear relationship was demonstrated between the amount of benzoic acid present in the stratum corneum at the end of a 30 min application period and the total amount absorbed over 4 days. We suggest that the influence of a vehicle on the overall absorption of a substance can therefore be predicted by simply measuring the amount present in the stratum corneum at the end of a 30 min application period.

In recent years, increasing attention has been paid to the influence that the components of a vehicle may have on enhancing or hindering the movement of a drug through the skin. Interest in biopharmaceutics, for instance, has stimulated the investigation of problems concerned with the formulation of vehicles for use in dermatology and with their effect on the activity of the drug. It is now well known that substances added to preparations as excipients, and other factors such as the physical form of the drug, cannot only affect their release and absorption, but also their action. The effects of vehicles have been reviewed in detail by several authors (Rothman, 1954; Barr, 1962; Idson, 1971; Idson, 1975). However, it seems that few techniques can be used routinely to elucidate rapidly the role that a vehicle, or a particular component in a vehicle, will play in the overall percutaneous absorption of a drug *in vivo*.

In previous studies in rats, using 10 different radiolabelled substances (Rougier *et al.*, 1983), and in humans, using increasing doses of benzoic acid (Dupuis *et al.*, 1984), we demonstrated a

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correlation between the amount of the test substance found in the stratum corneum at the end of a 30 min application, and the total amount absorbed over 4 days. We also showed that this relationship remained valid with longer exposure (up to 6 h) (Rougier *et al.*, 1985), thus establishing a predictive method for measuring *in vivo* percutaneous absorption in both rats and humans. The present study was carried out to determine whether this method could also be used to predict the effect of the vehicle on the degree of penetration of a test compound.

## METHODS

The influence of nine simple vehicles on the *in vivo* percutaneous absorption of U-$^{14}$C benzoic acid (The Radiochemical Centre, Amersham, U.K.) was studied in the hairless rat. The vehicles tested were mixtures of solvents, ethanol/water, methanol/water, ethyleneglycol/water and propyleneglycol/water, and pure solvents, glycerol and ethyleneglycol. The surface tension of some of these vehicles was increased by the addition of Triton X-100. The precise compositions of the vehicles tested are indicated in Table I.

A group of 12 Sprague Dawley female hairless rats, weighing 180–220 g, was used for each test.

20 $\mu$l of each vehicle, containing 200 nmol of benzoic acid, were applied to 1 cm$^2$ of dorsal skin of rats previously anaesthetized by an injection of $\gamma$butyrolactone (0·5 ml/kg i.p.). The application area was delimited by an open circular cell, attatched with silicone glue in order to prevent any loss of product. The application time was 30 min. After application, the treated area was washed twice with 300 $\mu$l of ethanol/water (95:5), rinsed twice with 300 $\mu$l distilled water and dried with a cotton tipped stick.

The 12 animals tested with each vehicle were then divided into two equal groups. In the first group, the stratum corneum in the treated area was removed by six strippings using 3 M invisible adhesive tape. Previous histological studies have shown that this technique removes most of the stratum corneum, which is formed by 7–8 cell layers (thickness 10–15 $\mu$m) in the hairless rat. The radioactivity in each strip was measured by liquid scintillation counting (Packard 460C, Packard Instruments, U.S.A.), after complete digestion of the keratinous material in Soluene 350 (Packard), and the addition of Dimilume 30 (Packard). The total amount of benzoic acid present in the stratum corneum at the end of the application was then determined by adding the amounts found in each strip.

The animals of the second group, fitted with a collar to prevent licking, were housed individually in metabolic cages for 4 days, with food (Extra Labo) and water *ad libitum*. The urinary excretion kinetics of benzoic acid were established by liquid scintillation counting of daily urine. The amount of benzoic acid excreted in the faeces over a period of 4 days was determined by liquid scintillation counting of the faeces, which were collected daily, pooled, lyophilized, homogenized and burnt in an Oxidizer 306 (Packard).

At the end of the experiment, the animals in the second group were sacrificed by cervical dislocation. A series of six tape strippings was performed on the treated area, in order to determine the amount of benzoic acid which had not penetrated the stratum corneum barrier within 4 days. Samples of the remaining skin (epidermis and dermis) were taken and were counted after digestion in Soluene 350. The bodies of the animals were lyophilized, homogenized and the total radioactivity after combustion, was measured by liquid scintillation counting. For each vehicle, the total amount of benzoic acid penetrating in 4 days was determined by adding the amounts found in the excreta (urine and faeces), in the epidermis and dermis of the treated area and in the whole animal body.

TABLE 1. Percutaneous absorption of benzoic acid from different vehicles in the hairless rat

| Vehicles | Maximum solubility of benzoic acid in the vehicle (mg/ml) | Total amount of benzoic acid 96 h after application ($nmol/cm^2$)* | | | | | Amount of benzoic acid in stratum corneum of treated area 30 min after application (nmol) |
|---|---|---|---|---|---|---|---|
| | | Urine | Faeces | Epidermis and dermis from treated area | Whole body | Total penetration | |
| 1 Propyleneglycol-triton X-100 | 219·5 | 0·81 | 0·32 | 0·03 | 0 | 1·15 | 0·73 |
| (90:10) | | (0·08) | (0·09) | (0·007) | | (0·12) | (0·07) |
| 2 Glycerol-triton X-100 (90:10) | 28·5 | 1·57 | 0·59 | 0·04 | 0 | 2·20 | 0·91 |
| | | (0·31) | (0·27) | (0·006) | | (0·44) | (0·10) |
| 3 Ethyleneglycol-triton X-100 | 130·5 | 4·18 | 0·38 | 0·12 | 0·02 | 4·71 | 3·10 |
| (90:10) | | (0·74) | (0·12) | (0·05) | (0·009) | (0·85) | (0·55) |
| 4 [Ethyleneglycol-triton X-100 | 6·5 | 4·45 | 0·48 | 0·16 | 0 | 5·10 | 3·91 |
| (90:10)]-[Water] [(40:60)] | | (0·48) | (0·14) | (0·04) | | (0·51) | (0·31) |
| 5 [Propyleneglycol-triton X-100 | 17·50 | 8·61 | 0·80 | 0·38 | 0·03 | 9·81 | 4·41 |
| (90:10)]-[Water] [(40:60)] | | (1·14) | (0·23) | (0·08) | (0·01) | (1·33) | (0·23) |
| 6 Ethanol-water (95:5) | 220 | 24·80 | 1·32 | 0·50 | 0·01 | 26·60 | 17·60 |
| | | (0·50) | (0·30) | (0·05) | (0·004) | (0·65) | (1·50) |
| 7 Methanol-water (40:60) | 19 | 30·46 | 2·50 | 0·88 | 0·18 | 34·02 | 18·68 |
| | | (2·93) | (0·47) | (0·10) | (0·10) | (3·01) | (1·86) |
| 8 Ethanol-water (60:40) | 148 | 43·42 | 3·98 | 1·02 | 0·09 | 48·52 | 23·56 |
| | | (3·97) | (0·36) | (0·27) | (0·03) | (3·57) | (3·43) |
| 9 Ethanol-water (40:60) | 40 | 49·56 | 3·59 | 0·78 | 0·15 | 54·08 | 28·39 |
| | | (4·95) | (0·39) | (0·12) | (0·02) | (5·21) | (1·73) |

* Values are mean (± SD) for six animals.

## RESULTS

The maximum solubility (mg/ml) of benzoic acid in each of the test vehicles, as determined by spectrophotometric assay, is shown in Table 1. The solubility of benzoic acid differed by a factor of 30 between the least and the most efficient solvent medium. Since benzoic acid is poorly soluble in aqueous media, the solubilizing power of the various vehicles for this compound decreased in relation to the amount of water, whatever the organic phase.

The distribution of benzoic acid between the urinary and faecal excretion routes, the body retention and the total absorption rate 96 h after application in the different vehicles are shown in Table 1. For a fixed concentration, the amounts of benzoic acid absorbed within 4 days varied by a factor of 50 with different vehicles.

The amounts of benzoic acid present in the stratum corneum at the end of the 30 min application period in relation to the vehicle used are indicated in Table 1. Figure 1 shows a linear correlation between these amounts and the quantities which had penetrated within 4 days ($r = 0{\cdot}997$, $P < 0{\cdot}001$).

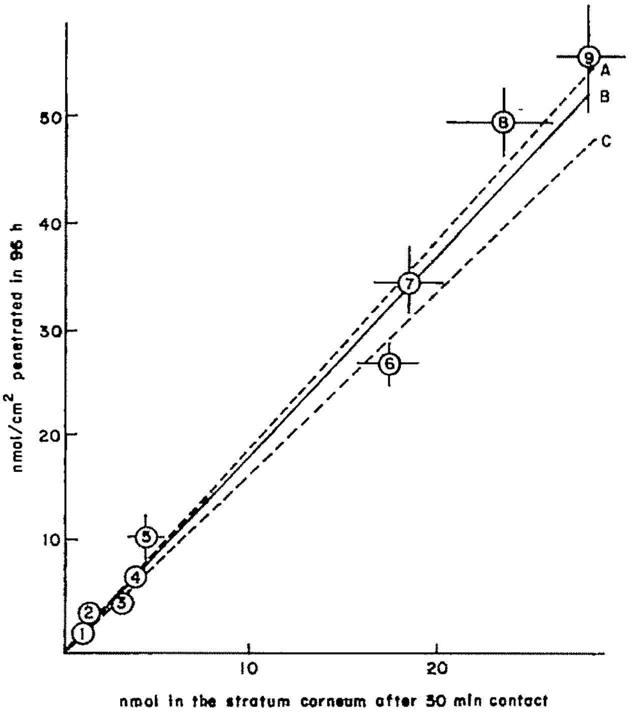

FIGURE 1. Correlation between the amount of benzoic acid absorbed after 96 h and the amount present in the stratum corneum after a 30 min application in different vehicles in the hairless rat. Points are means ± SD for groups of six animals. A (– – –): correlation obtained in humans with increasing doses of benzoic acid (Dupuis *et al.*, 1984); B (——): correlation obtained in the present study; C (– – –): correlation obtained in the hairless rat with 10 different compounds (Rougier *et al.*, 1983). Vehicles: (1) propyleneglycol-triton X-100 (90:10); (2) glycerol-triton X-100 (90:10); (3) ethyleneglycol-triton X-100 (90:10); (4) [(ethyleneglycol-triton X-100) (90:10)]- water (40:60); (5) [(propyleneglycol-triton X-100) (90:10)]- water (40:60); (6) ethanol-water (95:5); (7) methanol-water (40:60); (8) ethanol-water (60:40); (9) ethanol-water (40:60). Correlation coefficient = 0·997; $P < 0{\cdot}001$.

## DISCUSSION

Studies *in vivo* (Schutz, 1957) and *in vitro* (Blank & Scheuplein, 1964) have shown that the release of a substance can be increased by the selection of vehicles having a low affinity for that substance, or in which it is least soluble. By comparing the maximum solubility of benzoic acid to the penetration obtained we found that the greatest penetration was not obtained with the vehicle in which benzoic acid was least soluble, or *vice versa*. Whilst reduced absorption is related to increasing solubility in the case of ethanol-water vehicles (Nos. 6, 8 and 9; Table 1), it was not generally true for all vehicles.

It is possible, however, that the presence of surfactant in vehicles 1–5 meant that the benzoic acid was dissolved in micelles, rather than in a true simple solution. Thus, the benzoic acid may be associated with the surfactant, and hence unavailable, in a free form, for absorption.

It has been shown that applied vehicles have the potential to either increase or decrease the quantity of water in the stratum corneum and therefore to increase or decrease penetration (Barrett, Hadgraft & Sarkany, 1964; Shelley & Melton, 1949). Our results show that the penetration of benzoic acid was enhanced generally by increasing the water content of the vehicles (nos. 1 and 5 and nos. 6, 8 and 9; Table 1).

The amount of benzoic acid present in the stratum corneum at the end of a 30 min application may reflect the stratum corneum/vehicle partition coefficient of the compound, but could also depend on the rate of molecular movement (i.e. rate of entry) into the stratum corneum.

Our most important finding was that, independently of the vehicle tested and the solubility of the benzoic acid, there was a significant linear correlation between the amount of benzoic acid within the stratum corneum at the end of the 30 min application time and the total amount penetrating in 4 days. This relationship is similar to those obtained in the rat with several other compounds with different physicochemical properties (Rougier *et al.*, 1983; 1985), and in humans with increasing doses of benzoic acid (Dupuis *et al.*, 1984).

The influence of a vehicle on the overall percutaneous absorption of a compound can therefore be predicted by the simple measurement of the amount present in the stratum corneum at the end of a 30 min application time. Furthermore, due to the relatively large amounts of the substance present in the stratum corneum after application, this mildy invasive method should allow *in vivo* percutaneous absorption studies to be performed in both humans and animals, using sensitive but non-radioactive techniques.

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## *Case Reports*

# Metastasizing atrial myxoma: a case with multiple subcutaneous tumours

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## SUMMARY

We report a case with rapidly growing subcutaneous and intra-muscular tumours that were identical histopathologically to a pre-existing left atrial myxoma. No invasion of visceral organs or skeleton was found. This is a very rare case of metastasizing myxoma with wide dissemination and rapid metastastic growth, despite the benign appearance of the original tumour.

Myxoma is a tumour of primitive mesenchyme. It occurs in the heart, bone, skin, genito-urinary system and other soft tissues (Stout, 1984). Cardiac myxoma is the most common, and constitutes nearly 50% of primary cardiac tumours (Prichard, 1951). When it arises in the endocardium, embolization is the most important complication (Silverman, Olivin & Grattinger, 1962; New, Price & Carter, 1970), being a frequent cause of sudden death in this condition. Although histogenesis of cardiac myxoma remains controversial, myxoma has been recognized to be a true neoplasm (Fisher & Hellstrom, 1960; Stein *et al.*, 1969; Kelly & Bhagwat, 1972), rather than organized mural thrombi (Salyer, Page & Hutchins, 1975). There have been several reports of local recurrence (Dang, 1976; Read *et al.*, 1974) and of distant metastasis from an original cardiac myxoma, despite its benign histological appearance (Read *et al.*, 1974; In *et al.*, 1980).

## CASE REPORT

A 44-year-old man was first seen at Osaka University Hospital in December 1984 with multiple subcutaneous tumours on his trunk and lower extremities. He first noticed a small, non-tender tumour on his upper back in July 1984. Further tumours appeared and increased rapidly in size during the subsequent 6 months. Four months prior to the appearance of the first tumour, he had suffered from a right-sided hemiparesis and a moderate dysarthria. He was admitted to the

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National Cardiovascular Center, and underwent neurological and cardiovascular examinations. A left atrial tumour was detected by echocardiography and cardiac catheterization. Local excision of the tumour was carried out in April 1984 and a histopathological diagnosis of myxoma was made. It was suggested that his neurological signs were caused by emboli from the myxoma. The patient's neurological symptoms improved after excision of the myxoma.

On presentation at our clinic, the patient had multiple, non-tender, moderately firm masses on his back, shoulder, thighs, right leg and finger tip. They measured 1–5 cm in diameter. Most of the tumours were well circumscribed and freely mobile, but some of them appeared to invade deeply into the underlying tissues. The covering skin was smooth and of normal colouration. Biopsies were taken from the tumours on his upper back and scapular regions. The histopathological structure of these specimens resembled that of the original left atrial myxoma. The superficial lymph nodes were not enlarged. No invasion of visceral organs or skeleton was detected by X-ray, gastro-intestinal examination, CT scan or technetium bone scan. No signs of local recurrence could be detected by echocardiography.

### *Histopathology*

The original cardiac tumour was a gelatinous polypoid mass which had been attached to the left atrial appendage by a stalk. The tumour had nearly filled the atrial cavity. Microscopically, the tumour was composed of scattered stellate or spindle-shaped cells embedded in the abundant myxoid material (Fig. 1). These tumour cells lay either singly or in strands. Although the nuclei of the tumour cells were generally hyperchromatic, no pathological mitotic figures were present. In general, the tumour had a benign appearance histopathologically.

Three biopsies were taken from the subcutaneous tumours on the patient's upper back and the scapular regions. Two of them were located in the subcutaneous fat and one, in the scapular area, was embedded among the musculature. These tumours were sharply demarcated and multilobular, and the cut surface had a translucent, myxomatous appearance. No cysts,

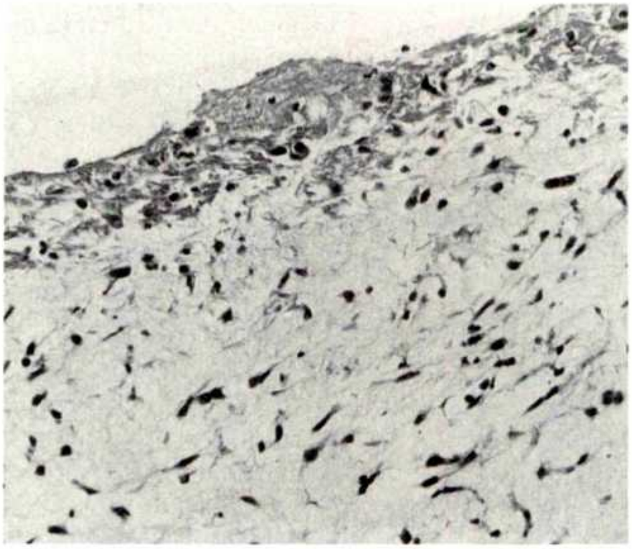

FIGURE 1. Histopathological features of the left atrial lesion. Scattered stellate, spindle or round tumour cells are embedded in myxomatous material (original × 250).

haemorrhage or necrosis were noted. There appeared to be no abnormalities in the overlying cutaneous tissue. Microscopically, these three specimens had identical structures and resembled the original left atrial tumour (Fig. 2). The cells possessed hyperchromatic nuclei and scanty cytoplasm, often extending along the reticulum fibres. Some vascular channel formation could be seen by strands of cells in the myxomatous material. Amorphous myxomatous material gave positive staining with Alcian blue. No storiform arrangement of the cells was observed. Lipoblasts were not seen. A section was stained for factor VIII-related antigen, using the peroxidase-antiperoxidase technique. A positive reaction for factor VIII-related antigen was found only in the cells which formed vessels embedded in the myxoid material.

## DISCUSSION

In the present case, all the histological features of the subcutaneous and intramuscular lesions were essentially the same as those of the left atrial myxoma. We examined carefully the possibility that the widespread subcutaneous lesions may be myxoid-type tumours of other mesenchymal origin. Stains for lipids using frozen sections of the tumour, were negative. The histological features of the subcutaneous tumours could be differentiated from myxoid liposarcoma because of the relative avascularity, much less cellularity and the lack of sundanophilic cytoplasm (Enterline *et al.*, 1960). They also differed from myxoid fibrosarcoma and malignant fibrous histiocytoma, because storiform arrangements of cells were not observed (Weiss & Enzinger, 1977). Both cardiac and widespread tumours contain very few tumour cells and their bulk is due to a mucinous matrix, presumably produced by the tumour cells. Although there have been several proposals as to the origin of myxoma cells, including endothelial cells (Morales *et al.*, 1981) and myoid-type cells, it seems to be a reasonable compromise to suggest that cells of myxoma are of primitive mesenchymal origin (Stein *et al.*, 1969; Ferrans & Roberts, 1973). In our case, factor VIII-related antigen was present only in the vasoformative cells,

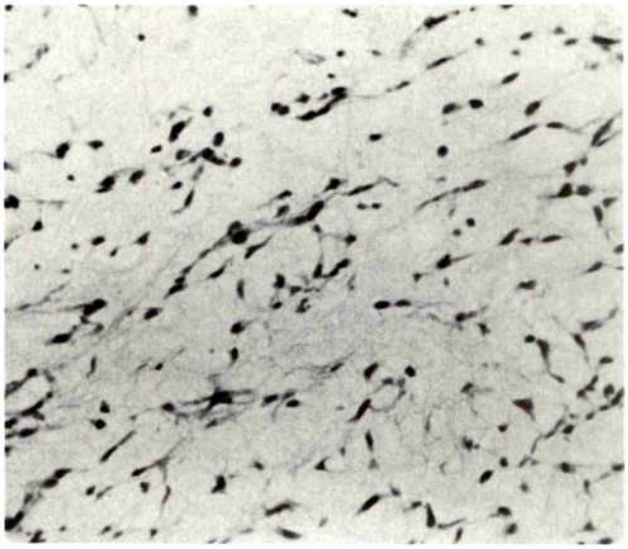

FIGURE 2. Biopsy specimen from a subcutaneous tumour on the upper back. Tumour cells lie singly or in strands (original × 250).

which made up the only organized structure identified (Jaffe, 1977). Usually, myxoma occurs in tissues where the structure is loose, and a good deal of fat is present. It often arises in the heart, bones, skin and genito-urinary system. It could be argued that our patient had suffered from a series of myxomas. However, it seems more plausible to consider the widespread lesions to be metastases from the original atrial myxoma.

There have been several reports that emboli coming from cardiac myxoma invade and destroy the arterial walls despite the benign appearance of the tumour (New *et al.*, 1970; Heath & Mackinnon, 1964). Our patient first suffered from cerebral emboli, but subsequently no tumorous invasion could be detected in the cerebral region by CT scan. It is reasonable to consider the subcutaneous tumours as metastases which occurred before removal of the left atrial myxoma.

This is a very rare case of myxoma and the patient is being followed up carefully.

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# A syndrome of keratosis palmo-plantaris congenita, pes planus, onychogryphosis, periodontosis, arachnodactyly and a peculiar acro-osteolysis

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## SUMMARY

A family with a symptom complex of pes planus, onychogryphosis, palmoplantar hyperkeratosis and periodontosis is reported. A detailed pedigree is given and the mode of inheritance is suggested.

A child with a symptom complex consisting of onychogryphosis, flat feet, palmo-plantar hyperkeratosis and periodontoclasia presented to us. This syndrome was partially described in four siblings in a family in Israel by Haim and Munk (1965). Flat feet were not mentioned in that report. Subsequently, four new cases were reported by Smith and Rosenzweig (1967) among relatives of the first family. All cases reported to date have been from amongst Jews who had emigrated to Israel from Cochin, India. Our case also belongs to this family and comes from the original family that has stayed behind in India.

A comprehensive survey has been conducted amongst all members of the family in Kerala and a total of four new cases have been examined in detail and investigated. This report, therefore, attempts to complete the description of this new syndrome, to shed fresh light on some aspects of the disease described earlier, and also to examine the geneology of the disorder.

## CASE REPORTS

### *Case 1*

The proband was $7\frac{1}{2}$ years old on presentation. He was born at term after a normal pregnancy. At birth the skin of his palms and soles was bright red in colour and appeared thin and glazed. By the age of 3 months the skin had become thick and scaly, and this roughened skin extended to the dorsum of the hands and feet, the extensor surfaces of the forearms up to the elbows and on the anterior aspect of the legs, extending to the thigh. During infancy the skin in the flexural aspects of the neck, axillae and groin was fissured, but this cleared when the child was one year old. The

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deciduous teeth were normal on eruption but by 3 years of age his gingiva was noted to be red, swollen and prone to bleeding. The teeth became loose and fell out by 4–5 years of age. At the time of examination eight permanent teeth had appeared.

On examination, he was of normal height and intelligence. He had bilateral flat feet, Broad Type B1, according to the classification of foot prints by Rose, Welton & Marshall (1985). Hyperkeratosis was seen over the entire palms and soles extending onto the dorsum of the hands and feet and circumferentially onto the forearms and legs for about 5 cm and further on the dorsal aspect of the forearms up to the elbows and the anterior aspects of shins, knees and thighs, stopping short of the groin by some 15 cm. His nails tended to bend inwards but were kept manicured close to the tips of the fingers.

*Dental findings*

$$\frac{\text{6xxxxx xxxxx6}}{\text{6xxx21 12xxx6}}$$

were the teeth present at the time of examination. Of the eight permanent teeth present, the lower anteriors were mobile. The gingiva was a dark pink. There was loss of stippling. The marginal gingiva was inflamed, swollen and bled on pressure. Dental X-ray showed marked resorption of alveolar bone, particularly around the root apices.

An X-ray of the feet showed flat feet (Fig. 1) while that of the hand was normal.

*Case 2*

Case 2 was a 5-year-old female relative of the proband. The history was similar except that the skin of her hands and legs was normal up to the age of 3 months when the hyperkeratosis appeared. Her teeth had erupted normally but started falling out by the age of 3 years.

*Dental findings*

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were present at the time of examination.

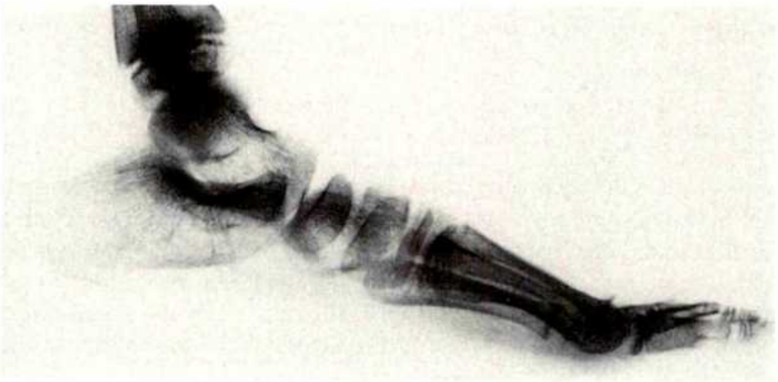

FIGURE 1. X-ray of foot lateral view (Case 1), showing pes planus.

The gingival margin was red and bled on pressure, in spite of good oral hygiene. She too had onychogryphosis and flat feet. X-rays of the skull, hands and feet were normal except for flat feet. The metacarpal index was 7·3 (normal below 8 and arachnodactyly above 8·4).

### *Case 3*

Case 3 was a 42-year-old aunt of these two children. She is married and has two children of her own, a boy aged 9 years and a girl of 7½ years, both of whom are normal. She developed her skin lesions at the age of 3 months. She too had the picture of early shedding of teeth preceded by gingival inflamation. She was edentulous by her late teens. At the age of 15 years she was hospitalized once for a liver abscess.

She had marked hyperkeratosis of her palms and feet, flat feet (also Broad Type B1) and onychogryphosis. An X-ray of the skull did not show any abnormality. An X-ray of the hand showed a metacarpal index of 9·7, a high arachnodactylic value (Fig. 2). The terminal phalanges of fingers 2–5 showed marked thinning and the tapering claw-like volar bend described by Haim and Munk (1965) (Fig. 3).

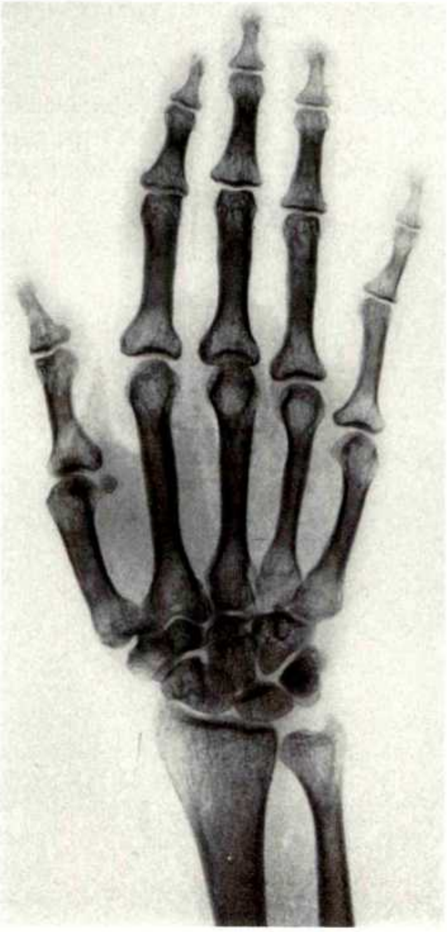

FIGURE 2. X-ray of hand (Case 3), showing arachnodactyly and high metacarpal index.

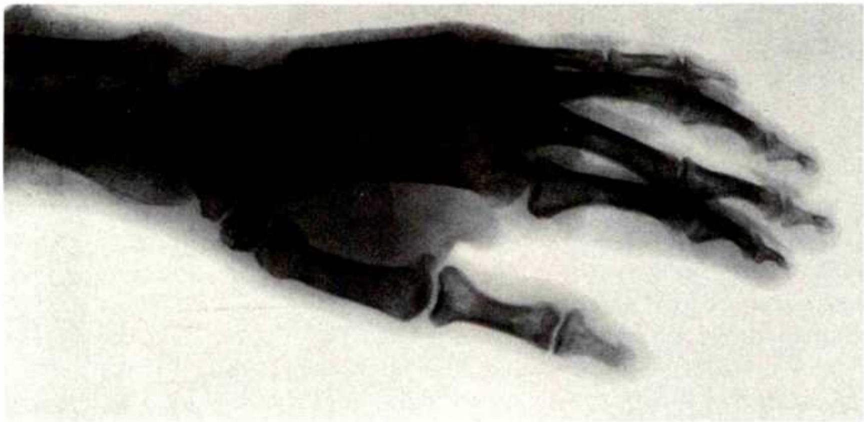

FIGURE 3. X-ray of hand, oblique view (Case 3), showing acro-osteolysis and the claw-like volar surface.

Her serum bilirubin, AST, ALT, alkaline phosphatase, total serum protein, albumin and globulin, serum calcium and serum electrophoretic pattern were all within normal limits.

*Case 4*

Case 4 was the 34-year-old brother of Case 3. He is unmarried. The history of skin lesions and periodontal changes were essentially the same as Case 3. At the time of examination he was edentulous. He too had flat feet and marked onychogryposis (Fig. 4). An X-ray of the hand

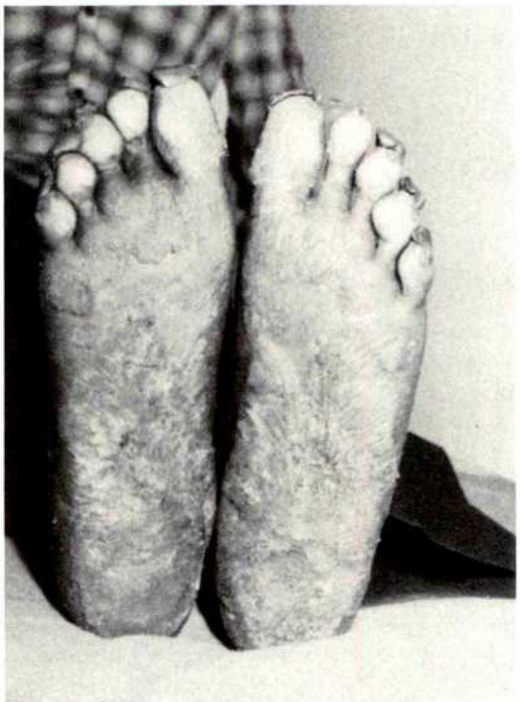

FIGURE 4. Photograph of both feet (Case 4) showing plantar keratosis and onychogryphosis.

showed a metacarpal index of 8·7 with the terminal phalanges showing the claw-like bend. A skin biopsy from the thenar eminence of the palm revealed hyperkeratosis, hypergranulosis and acanthosis while the basal layer was intact. His serum bilirubin, AST, ALT, alkaline phosphatase, serum calcium, total serum proteins, albumin and globulin and serum electrophoretic pattern were all within normal limits.

## DISCUSSION

The features of this syndrome are the sequential appearance of hyperkeratosis, primarily of the hands and feet, periodontoclasia and premature loss of teeth, flat feet, onychogryphosis, arachnodactyly and acro-osteolysis. No variability in expression of the syndrome seems to exist, and all adult affected family members seen exhibited all the manifestations. To date, this syndrome has not been reported in any other family.

Papillon and Lefevre (1924) described a syndrome of hyperkeratosis palmoplantaris and periodontoclasia. Gorlin, Sendano and Anderson (1964) described a case of Papillon-Lefevre syndrome with onychogryphosis of the toe nails, but in a review of the disorder by the same author he notes that the nails are rarely, if ever involved (Gorlin, Pindborg & Cohen, 1976).

Keratosis palmo-plantaris, periodontosis, arachnodactyly and onychogryphosis in association with acro-osteolysis was first described by Haim and Munk (1965). No mention is made in this report about the presence of flat feet.

Pes-planus was seen in all four cases examined by us. Flat feet in these cases were diagnosed from X-rays and also by the foot printing method described by Rose *et al.* (1985). While flat feet were seen in all members of the family with palmoplantar keratosis, they were not seen in other members who were not affected. Personal communication with Professor Friedman, the successor of the late Professor Haim, has established that four cases of the syndrome in Israel also have flat feet. Two of these cases were described in the original case report by Professor Haim.

The radiological appearance of progressive thinning of the bone of the terminal phalanx to form a claw-like volar curve was seen in the two older cases reported here. However, it was not seen in the two children of 7 and 5 years old. Haim and Munk (1965) reported this in their cases aged 17, 15 and 12 years, but this was not seen in their 7-year-old case, suggesting that this deformity is due to bone absorption which is complete by about 12 years of age and not due to a congenital or developmental disorder.

A high metacarpal index was seen in both the adults described in this report and was present in the three older children reported by Haim and Munk (1965). The metacarpal index was normal in the children reported here and also in the 7-year-old reported by Haim and Munk.

Haim and Munk reported recurrent abscess formation amongst their cases, attributable to chronic inflammation of the skin. Case 3 reported here had a liver abscess at the age of 15 years, but none of the others showed an excessive predisposition to infection.

The four cases reported here are related to the four examined by Haim and Munk and to the four new cases described by Smith and Rosenzweig (1967). All these cases share the same family tree (Fig. 5) and belong to a small community of Jews from Kerala. There is a high incidence of consanguineous marriage in this small community. The fact that five reported cases are male and seven female suggests an autosomal mode of inheritance. None of the parents of the children reported here is affected by the disorder and this precludes a dominant gene. Thus, an autosomal recessive gene seems most likely.

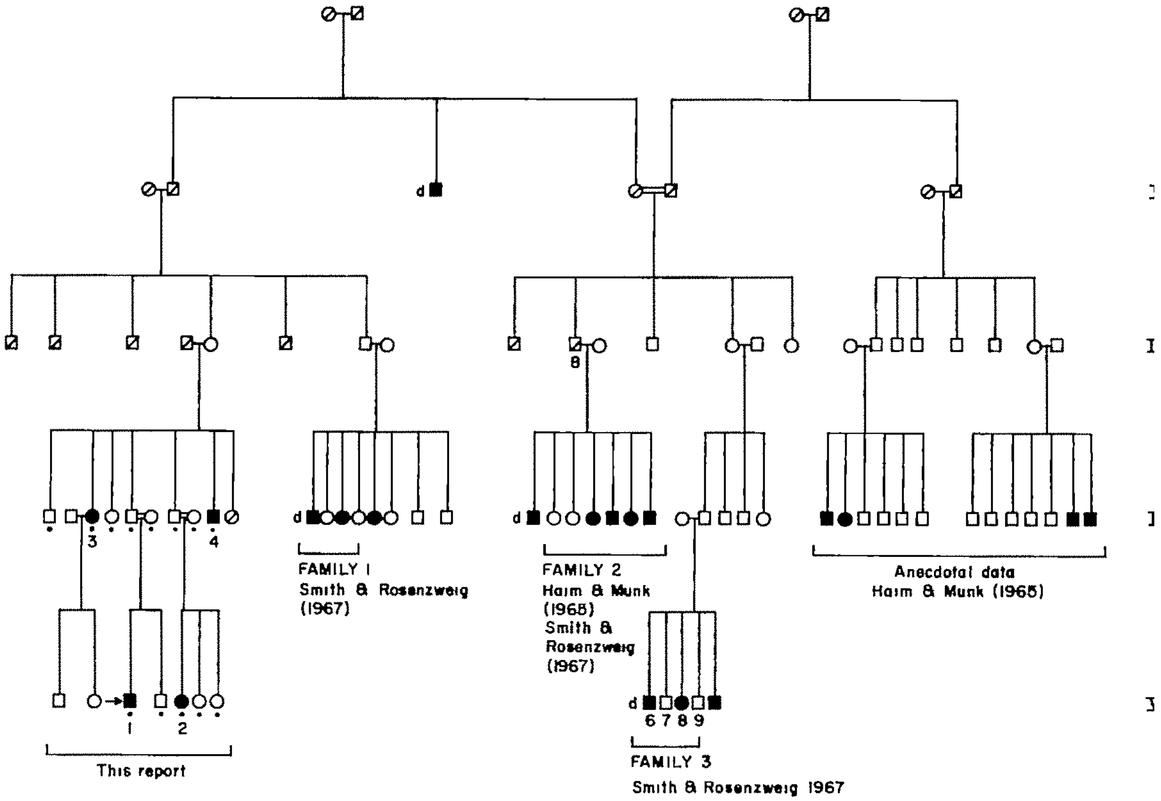

FIGURE 5. Family pedigree chart. →■ proband; ■ affected, examined; ○ normal, examined; □ male; ○ female; d ■ affected, not alive; ⊘ normal, not alive; ○–□ marriage; ○=□ consanguinity.

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*British Journal of Dermatology* (1986) **115**, 249–255.

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## Degos' disease

MADAM, We noted with interest the recent letter by Habbema, Kisch and Starink (*British Journal of Dermatology*, 1986, **114**, 134), in which familial malignant atrophic papulosis (Degos' disease) was reported. The authors state that their report brings the number of families with Degos' disease to four. We would point out that we reported a fifth family similarly affected in 1984 (Newton & Black, 1984). It is of particular interest that in our family, as in that reported by Habbema *et al.*, the course of the disease was benign, in marked contrast to the original reported cases of this disease.

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## Cyclopsorin A and psoriasis

MADAM, In 1979, Mueller and Herman reported the dramatic response of four cases of severe psoriasis to oral cyclosporin A (CyA). Harper, Keat and Staughton (1984) described the rapid remission of erythrodermic psoriasis following oral CyA in a patient unresponsive to PUVA, methotrexate, etretinate, and razoxane, and suggested that CyA may prove a useful drug for the treatment of severe psoriasis unresponsive to conventional therapy. Van Hoof, Leunissen and Staak (1985) suggested that CyA may be a useful systemic agent in the management of severe psoriasis. Aldridge *et al.* (1985) have shown that topical CyA has pharmacological activity in the rat, and have suggested that it may be an effective treatment of psoriasis in man.

It is in the context of these favourable accounts of CyA and psoriasis that I wish to report a patient in whom CyA appeared to induce the development of psoriasis.

A 55-year-old man under the care of the Liver Unit at King's College Hospital, for histologically proven primary biliary cirrhosis (PBC) agreed to enter a double-blind trial of oral CyA versus placebo. The rationale for this study is that PBC is believed to have many pathological features in common with chronic graft versus host disease, a disorder on which CyA is thought to have a favourable influence. Treatment was started in April 1985 at a dosage of 2 ml per day of CyA/placebo solution (i.e. 200 mg CyA); he was on no other drug therapy. In May 1985, the patient developed typical plaque psoriasis, having had no past or family history of this disorder, and was referred to the Department of Dermatology. In August 1985, the dosage of CyA/placebo was increased to 2·5 ml, and by October 1985 the psoriasis was considerably more extensive and resistant to dithranol and potent corticosteroid therapy. At this point, it was decided to break the trial code, and it was found that the patient had been taking the active drug. In the light of the severe and resistant nature of the psoriasis, and the time course of its onset in relation to the CyA therapy, the patient was then withdrawn from the study. Within 2 months the psoriasis had gone into complete spontaneous remission.

Although it is possible that the relationship between the psoriasis and CyA therapy in this patient was entirely fortuitous, the time courses of onset and remission do suggest a causal connection. This case should act as a caveat for those considering employing CyA as a therapeutic agent in psoriasis.

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## Elevated serum amylase in toxic epidermal necrolysis

MADAM, We were very interested in the letter by Tatnall, Dodd and Sarkany (*British Journal of Dermatology* 1985, **113**, 629), in which they reported a patient with tetracycline-induced toxic epidermal necrolysis (TEN) showing elevated serum amylase levels which initially caused a diagnostic problem concerning pancreatitis. We had a similar experience with a case which was reported as ampicillin-induced TEN (Tagami *et al.*, 1983).

The isoamylase studies in the case reported by Tatnall *et al.* demonstrated that the excess amylase was salivary in origin, suggesting that the patient had a subclinical parotitis. Hence, they speculated on the possibility of specific involvement of the salivary glands in TEN, also taking into consideration the recent report by Roujeau *et al.* (1985) of Sjogren-like syndrome following TEN. In our case, we suspected pancreatitis because of elevated serum and urinary amylase levels, the dominant isozyme being demonstrated to be of pancreatic origin; levels returned to normal within a week. Our case also showed increased levels of other serum enzymes, including creatine phosphokinase, lactic dehydrogenase with a multiple isozyme profile, SGOT, SGPT, and alkaline phosphatase, suggesting multi-system involvement. Thus, while Tatnall *et al.* emphasize only the possible salivary involvement in TEN, we suggest that the pancreas could also be a target organ in drug-induced TEN, with a resultant increase in serum amylase levels.

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REFERENCES

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## Reply

MADAM, We are interested to read that other authors have encountered a raised serum amylase in toxic epidermal necrolysis. Tagami and Iwatsuki speculated that their patient had pancreatitis because the principle isoamylase that was elevated was pancreatic, although, apparently their patient had no symptoms to suggest this diagnosis. An increase in serum pancreatic isoamylase is insufficient evidence on which to base a diagnosis of pancreatitis, as pancreatic amylase may be elevated in other conditions. Similarly, although we were able to demonstrate an elevated salivary isoamylase in our patient, she had no symptoms referrable to the salivary glands. Although salivary isoamylase is produced by many organs, it seems reasonable to assume that, with severe oral involvement in toxic epidermal necrolysis, the salivary glands might also be affected.

In each of these cases it is difficult to be certain of the underlying pathological processes which have given rise to the elevated serum amylase. We agree with Tagami and Iwatsuki that more widespread organ involvement may be associated with toxic epidermal necrolysis, and perhaps future studies will elucidate this problem.

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## Anthralin inflammation: lack of effect of topical thiocarbamide drugs

MADAM, Following our report of the inhibitory effect of free radical scavengers on anthralin inflammation (Finnen, Lawrence & Shuster, 1984), Ferguson *et al.* (1985) reported an inhibitory effect of the hydroxyl radical (·OH) scavengers methimazole and 2,mercaptobenzimidazole on UV-induced skin erythema. These authors suggested that the lack of inhibitory effect of mannitol, another ·OH scavenger used in our study (Finnen *et al.*, 1984), may have been due to poor penetration of aqueous mannitol, and took up the suggestion of Whitefield, Leeder and Mosedale (1985) that the action of α-tocopherol, retinol and butylated hydroxyanisole was due to oxidation of anthralin by them and not a free radical action. We are now able to refute both these suggestions.

We have recently been able to examine the unpublished data (Whitefield, Leeder & Mosedale, personal communication) on which the claim was based that α-tocopherol reduces anthralin inflammation by promoting its oxidative destruction (Whitefield *et al.*, 1985). It shows that such destruction is slow: after 28 days there was a 66% decrease in recoverable anthralin, at 5 days it was 28% and at 1 day only 11%. Thus, at 48 h, the time we found anthralin inflammation was completely aborted by α-tocopherol, there would have been only a small (<20%) loss of anthralin. We can therefore conclude that tocopherol prevents development of anthralin inflammation by its free radical scavenging effect and not by chemical inactivation.

To examine the suggestion (Ferguson *et al.*, 1985) that our negative findings with an ·OH scavenger were due to poor penetration, we examined the effect of methimazole and 2,mercaptobenzimidazole on anthralin inflammation using n-butanol as a solvent from which penetration has been demonstrated (Ferguson *et al.*, 1985). Anthralin inflammation was produced by pipetting anthralin BP 40 μg/μl chloroform onto clinically normal flexor forearm skin of six healthy volunteers and three patients with psoriasis (five female, four male, mean age 30 years, range 21–70). At three sites this was followed by 20 μl of freshly prepared supersaturated solutions of methimazole and 2,mercaptobenzimidazole (Aldrich Chemicals, U.K.) in n-butanol and by the solvent n-butanol alone. At two other sites, anthralin and n-butanol were applied singly. The areas were covered with parafilm and micropore tape for 6 h and the inflammation was measured as increase in skinfold thickness 48 h after anthralin application using Harpenden calipers (Lawrence & Shuster, 1985). Results were analysed using Dunnett's test for multiple comparisons with a single mean.

The results (Fig.) show that neither ·OH scavenger had any effect on anthralin inflammation ($P > 0{\cdot}05$), despite their reported effect on the UV response (Ferguson *et al.*, 1985). This supports the earlier finding that anthralin inflammation is not mediated by the ·OH radical (Finnen *et al.*, 1984). Thus, abolition of

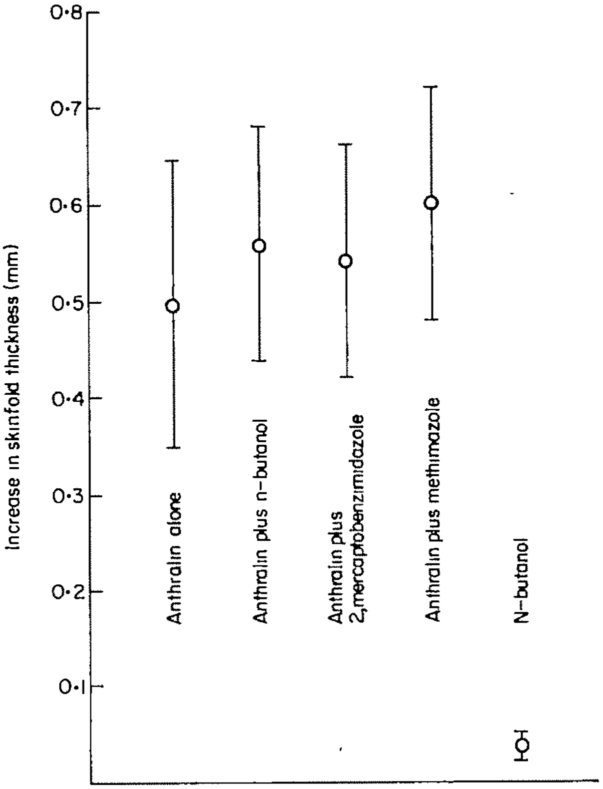

FIGURE. Effect of 2,mercaptobenzimidazole and methimazole on anthralin inflammatory oedema. Mean ± SEM ($n=9$).

anthralin inflammation by the free radical scavengers tocopherol, retinol and butylated hydroxyanisol indicates that anthralin inflammation is mediated by another active oxygen species or anthralin-free radicals.

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## Haematological consequences of methotrexate therapy

MADAM, The letters from Dr Fulton and Drs Oxholm and Thomsen (*British Journal of Dermatology*, 1986, **114**, 267 and 268) have stimulated me to comment on the earlier letter from Dr Dodd and his colleagues (*British Journal of Dermatology*, 1985, **112**, 630).

It is intriguing how the demise of razoxane, the carcinogenicity of PUVA and a certain disappointment with etretinate have focused attention back to methotrexate, so that we are having to relearn some of the lessons of the 1960s.

Dr Dodd and his colleagues suggested without qualification that methotrexate should be withdrawn if the MCV rises above 106 fl. I cannot agree with this advice which, if followed, would put some patients in the far greater danger of erythrodermic relapse of their psoriasis and potentially fatal metabolic sequelae in the elderly with cardio-vascular disability. Better management has always been to continue the methotrexate but to add folic acid 5 mg orally twice or thrice weekly. Folinic acid is unnecessary. The MCV will come down and anaemia will be prevented.

Even if macrocytic anaemia has developed—and I agree that it can do so suddenly after years of uneventful therapy—the anaemia can usually be corrected with small oral doses of folic acid without stopping methotrexate and without risking severe consequent relapse of the psoriasis.

Various factors can contribute to the folate deficiency and Dr Fulton has drawn attention to one of them. I agree with the advice given by Drs Oxholm and Thomsen, which was the management recommended in Rook's *Textbook of Dermatology* in both the second (1973) and third (1979) editions (p. 1344).

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## Amiodarone-induced cutaneous photosensitivity

MADAM, The clinical response of patients with amiodarone-induced cutaneous photosensitivity suggests a phototoxic, rather than a photoallergic, reaction and a recent clinical and *in vitro* study by Ferguson *et al.* (*British Journal of Dermatology*, 1985, **113**, 537) has supported this view. In their study of 12 patients receiving amiodarone (100–400 mg/day) nine patients exhibited abnormal immediate erythema after exposure to ultraviolet radiation between 335–400 nm, and 10 patients exhibited abnormal delayed erythema after exposure to 335–460 nm. These results corresponded to the author's clinical observations that wavelengths of ultraviolet light transmitted through window glass and from a UVA sunbed produced unpleasant burning or prickly sensations in about 50% of patients taking amiodarone.

We have been treating a 47-year-old male psoriatic patient with PUVA therapy for 2 years. During this time, he has been taking amiodarone 400 mg daily for a supraventricular cardiac arrhythmia. He has received a total cumulative dose of 2000 J/cm$^2$ but to date, rather surprisingly, has been completely free from any photosensitivity side-effect of amiodarone. He takes 40 mg of methoxypsoralen before PUVA treatment and has developed a normal PUVA tan. At no time has he experienced symptoms of burning or prickling of the skin.

In view of the well recognized photosensitivity associated with amiodarone, the patient's PUVA treatment was introduced cautiously with the dose of UVA increasing half as quickly as for a comparable patient with skin type III.

There is no information available from either the drug manufacturer or the Committee on Safety of Medicines relating to concurrent treatment with amiodarone and PUVA. However, this case demonstrates that amiodarone need not be an absolute contra-indication to PUVA therapy.

Our patient had considerable exposure to UVA light over a 2-year period, and an abnormal photosensitive reaction could well have been expected. It may be that the PUVA-induced tan reduced the amount of UVA light penetrating to the dermis, but the tanning took some weeks to develop and the patient did not complain of symptoms during this period. Alternatively, PUVA therapy may have modulated the patient's ability to produce a photosensitivity reaction, perhaps in a similar way to that used therapeutically to desensitize patients with polymorphous light eruption. Formal clinical trials would be required to address these points.

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## Reply

Madam, The most likely reason in this interesting PUVA patient for the apparent freedom from amiodarone phototoxcity is that the treatment dose of UVA in PUVA has fallen short of the dose required for clinical phototoxicity. In our study, the required dose of monochromator irradiation at 365 ± 30 nm was in the region of 5–10 J/cm$^2$, whereas in PUVA treatments a typical starting dose may be 1–3 J/cm$^2$.

An alternative explanation is that the pharmacological handling of amiodarone and its phototoxic metabolite by this patient is rapid, a possible reason for those recognized patients who, despite low skin types and high dosage, do not develop phototoxicity (for example, our Case 10).

In Dundee, it is our experience with PUVA that recognized phototoxic drugs, in particular tetracyclines, thiazides, quinine and oestrogens, when taken at normal therapeutic dosages, do not either lower the pre-PUVA minimal phototoxic dosage (MPD) or affect the subsequent phototoxic burn rate. Accordingly, it is our practice not to stop essential phototoxic medication prior to PUVA but to take the precaution of assessing such patients pre-PUVA with an MPD. These observations are in line with other workers (Stern *et al.*, 1980) although they, in a large study, while demonstrating no difference between burning episodes in phototoxic medication takers during the clearing phase of PUVA and non-drug taking PUVA patients, did report that, during the maintenance period, elderly patients on photoactive drugs had a slight increased likelihood of discontinuing PUVA because of burns.

That such medication is safe when exposed to high intensity UVA such as is used in sunbeds is, of course, a different matter entirely (Rosen & Swanbeck, 1981). It is likely, as in our Case 6, that the much higher UVA dosages used will induce clinical phototoxicity.

Dr Boyle's case illustrated the important point that patients taking essential phototoxic medication need not be excluded from PUVA.

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## Arterial lesions of the lower lip

Madam, Marshall and Leppard (*British Journal of Dermatology*, 1985, **113**, 757) describe lip ulceration and simulation of cancer by a calibre-persistent branch of the inferior labial artery. They mention previous work by Miko, Adler and Endes (1980) and by Miko, Molnar and Vereckei (1983).

May I draw your attention to the report by Howell and Freeman (1973) concerning nodules of the lower lip caused by a cylindrical dilation of the inferior labial artery. The artery was tortuous and more superficially located than usual, but otherwise its anatomical course was normal. Howell and Freeman's advice was to examine the immediate vicinity of the lesions for tortuous superficial vessels and for pulsation. I wonder is this advice not also valid for the kind of cases described by Marshall and Leppard and by Miko *et al.*?

Nodules and ulcers that mimic skin cancers may be caused by the inferior labial artery itself, as well as by wide-calibre branches that run through the orbicularis oris muscle.

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MIKO, T., MOLNAR, P. & VERECKEI, L. (1983) Interrelationships of calibre-persistent artery, chronic ulcer and squamous cancer of the lower lip. *Histopathology*, 7, 595.

## Reply

MADAM, Dr Howell was kind enough to send me a copy of his report following publication of our article. Clearly, the lesion described by Howell and Freeman is very similar to ours, in that an artery of inappropriately large calibre is present at a very superficial location. It is possible that the nodular lesion is the precursor of the ulcer, although this seems unlikely, as it is hard to see how the artery could sink from its position within the nodule to the base of the ulcer, at least without torrential haemorrhage. Possibly, the lesions represent different sequelae of the same underlying pathology. It is interesting that the histological appearance of the arteries is very similar in the two lesions.

Howell and Freeman's article is also important because they argue strongly against surgical excision. This would be a bold approach to the ulcer lesion, especially since Miko, Molnar and Vereckei (1983) have described carcinoma arising in association with calibre-persistent artery. It is worth attempting to palpate the artery in our lesion also, as Dr Frumkin suggests. However, the superficial part of the artery could be very focal in nature and palpation is not likely to provide a diagnosis of sufficient confidence to leave well alone.

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*British Journal of Dermatology* (1986) 115, 256–257.

# *Book Reviews*

**Précis de Dermatologie et Vénéréologie.** J-H.SAURAT, E.GROSSHANS, P.LAUGIER and J-M. LACHAPELLE. Paris: Masson. Pp. 661. Price

The *Précis* is hallmarked with the stamp of excellence that has come to be expected from Saurat and colleagues. There are four editors and 94 contributors. Each French-speaking dermatological teaching centre is represented and there are two English-speaking authors, one German and one Spaniard.

Well presented, on good quality paper and with an attractive type face, the *Précis* is right up-to-date with references as recent as October 1985. There is an excellent index, which includes entries for most eponyms and the minimum of annoying cross-referencing. The illustrations are good and include welcome pictures of angioid streaks, Gottron's papules, Bazex's syndrome and Blaschko's lines. There are up-to-date sections on the pathophysiology of keratinization, the dermo-epidermal junction and the use of immunoblotting in the diagnosis of bullous diseases. Drs Baran and Dawber have contributed a useful glossary of nail language. Each new textbook should describe at least one new syndrome and this is the first to mention the frontal subaponeurotic lipoma (Grosshans). This common tumour affects males after the age of 30 and presents as a symptomless smooth-domed swelling of the forehead. Surgical excision from the subaponeurotic plane is not for 'dermatological surgeons', only four out of 20 operations were successful in one series. Cary Grant numbers amongst the rich and famous who have had a Grosshans tumour.

On some subjects more detail might have been preferred. For instance, there is only brief mention of dithranol (a reflection of French prescribing habits) and the biological and scientific strength of French dermatology is not given the prominence it deserves. Nevertheless, if in 661 pages the *Précis* cannot be as comprehensive as Rook, Wilkinson and Ebling, it is easily the equal of texts of comparable length. With 2500 dermatologists in France the *Précis* should sell well.

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R.C.D.STAUGHTON

**Therapeutic Photomedicine.** Edited by H.HONIGSMANN and G.STINGL. Karger. Price £71·90.

The book, an attractively presented, multi-author volume in the *Current Problems in Dermatology* series, is based on a clinically-oriented symposium of the European Society for Dermatological Research (ESDR) held in Baden and Vienna in 1985. Most of the authors are internationally acclaimed authorities actively engaged in their respective fields. Predictably, the quality of the chapters varies, but the best are excellent.

Almost all aspects of photomedicine, including the use of lasers, are reviewed; one exception is the use of visible light for hyperbilirubinemia. The relatively new discipline of photoimmunology is also comprehensively covered by its pioneers including Dr Margaret Kripke, who summarizes *The First Decade* of what is certain to be a continuously expanding and important field. A large section is devoted to clinical phototherapy and photochemotherapy, including a critical appraisal of combination phototherapy for psoriasis. A perhaps controversial inclusion is that of the late Professor Greiter on the physiological effects of UVR, such as changes in serum levels of adrenalin, noradrenalin and $\beta$-endorphin. This is an area which in general orthodox Western photobiology has chosen so far to ignore. The concluding chapter is a review of experimental photocarcinogenesis and its relationship to human risk evaluation. Photoageing is not discussed.

In a number of chapters there is a welcome emphasis on molecular and mechanistic aspects, especially with the furocoumarins in chapters by Gasparro *et al.* and Dall'Acqua. There are some errors, e.g. the incorrect use of the term photodynamic on page 222. Readers are referred to page 140.

Overall, I liked this book. It is a valuable, up-to-date reference text. However, at £71·90, *Therapeutic Photomedicine* is probably for the specialist or the library rather than for the general dermatologist.

A.YOUNG

## Book Received

**Practical Pediatric Dermatology.** W.L.WESTON. 2nd edition. Boston: Little Brown. Pp. 410. Price £36.45.

1st edition reviewed in *British Journal of Dermatology*, 1980, Vol. 102, p. 753.

*British Journal of Dermatology* (1986) **115**, 258.

# *News and Notices*

## Introductory Course on the Biology of the Skin, Cambridge, December 1986

The annual introductory course 'The Biology of the Skin' will be held in Fitzwilliam College, Cambridge from 8 to 12 December 1986. This course is primarily for registrars and postgraduate students at an early stage of their training in dermatology, but is open to other interested practitioners. The closing date for applications is 17 November 1986. Further information and an application form may be obtained from: the Conference Organiser, Postgraduate Medical Centre, Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K. Telephone: Cambridge (0223) 212216.

## Institute of Dermatology – Fifth Course for Consultants and Senior Registrars, 1987

The above course, entitled Skin Cancer: Diagnosis and Treatment, will be held on the 6–8 February 1987 at the Unicorn Hotel, Bristol. For further details please contact the course director Professor M.W.Greaves, or his administrative assistant Mrs Rosemary Barton, at the Institute of Dermatology, Homerton Grove, London E9 6BX, U.K. Telephone: 01 985 7061.

## Fellowship of the Royal College of Physicians of London

The following have recently been elected to the Fellowship of the Royal College of Physicians of London: Drs D.Desmond Burrows, Dorothy M.France, Peter G.Goodwin, Cameron C.T.Kennedy, Arthur G.Knight, Barbara Joan Leppard, Ashley V.Levantine, David H.McGibbon, Leslie G.Mullard, Marguerite M.Roberts and Richard C.D.Staughton.

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**Manuscripts.** Three copies of all material for publication should be sent to the Editor, Professor Rona M.MacKie, Dermatology Department, Glasgow University, Glasgow G12 8QQ. Manuscripts should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

**Abbreviations and units.** Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

| | | | | | |
|---|---|---|---|---|---|
| gram(s) | g | minute(s) | min | molar | mol/l |
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| milligram(s) ($10^{-3}$g) | mg | second(s) | s | gravitational acceleration | ***g*** |
| microgram(s) ($10^{-6}$g) | μg | cubic millimetre(s) | $mm^3$ | micrometre(s) | μm |
| nanogram(s) ($10^{-9}$g) | ng | millimetre(s) | mm | per cent | % |
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# British Journal of Dermatology, Volume 115, Number 2, August 1986

CONTENTS PAGE

### Invited Editorial

The electron microscope and the antibody: trends in the diagnosis of epidermolysis bullosa 123
CHRISTINE J.SKERROW

### Laboratory and Clinical Investigations

Identification of an epidermal basement membrane defect in recessive forms of dystrophic epidermolysis bullosa by LH 7:2 monoclonal antibody: use in diagnosis 125
A.H.M.HEAGERTY, A.R.KENNEDY, I.M.LEIGH, P.PURKIS AND R.A.J.EADY
Secretion of plasminogen activator inhibitor by cultured human epidermal cells 133
K.HASHIMOTO, K.NISHIOKA, I.KATAYAMA AND K.YOSHIKAWA
Contraction of collagen lattices by fibroblasts from patients and animals with heritable disorders of connective tissue 139
P.DELVOYE, CORNELIA MAUCH, T.KRIEG AND C.M.LAPIERE
Elevated thymosin alpha-1 levels in Brazilian pemphigus foliaceus 147
J.T.ROSCOE, P.H.NAYLOR, L.A.DIAZ, R.S.LABIB, H.P.PATEL, A.L.GOLDSTEIN, S.A.P.SAMPAIO AND G.J.ANHALT
An assessment of the diagnostic value of the monoclonal antibodies Leu 8, OKT9, OKT10 and Ki67 in cutaneous lymphocytic infiltrates 151
MARLYN L.TURBITT AND RONA M.MACKIE
Leukapheresis in the treatment of cutaneous T-cell lymphomas 159
S.V.BELTER, J.KNOP, K.BRÜSKE AND C.SORG
Patterns of dermatology referrals in a general hospital 167
N.HARDWICK AND NORMA SAXE
Brachioradial pruritus: a tropical dermopathy 177
PATRICIA J.WALCYK AND D.J.ELPERN
Elastase, a marker for neutrophils in skin infiltrates 181
A.M.LAMMERS, P.C.M.VAN DE KERKHOF, J.SCHALWIJK AND P.D.MIER
Immunohistochemical study of lysozyme in lupus miliaris disseminatus faciei 187
K.MIHARA AND M.ISODA
Hyper-induction of skin ornithine decarboxylase activity in psoriatic patients 193
M.BOUCLIER, P.ELBAZE, G.MILANO, B.SHROOT AND J-P.ORTONNE
The autoimmune background in lichen planus 199
D.SHUTTLEWORTH, R.A.C.GRAHAM-BROWN AND A.C.CAMPBELL
Plasminogen activator secreted by cultured human melanocytes 205
K.HASHIMOTO, T.HORIKOSHI, K.NISHIOKA, K.YOSHIKAWA AND D.M.CARTER

### Pharmacology and Treatment

Treatment of chronic discoid lupus erythematosus with an oral gold compound (auranofin) 211
K.DALZIEL, G.GOING, P.H.CARTWRIGHT, R.MARKS, G.W.BEVERIDGE AND N.R.ROWELL
Itraconazole therapy in pityriasis versicolor 217
A.DEL PALACIO HERNANZ, J.FRIAS-INIESTA, O.GONZALEZ-VALLE, M.BORGERS, J.VAN CUTSEM AND G.CAUWENBERGH
Oral spironolactone: an effective treatment for acne vulgaris in women 227
M.F.MUHLEMANN, G.D.CARTER, J.J.CREAM AND P.WISE
The measurement of the stratum corneum reservoir: a simple method to predict the influence of vehicles on *in vivo* percutaneous absorption 233
D.DUPUIS, A.ROUGIER, R.ROGUET AND CLAIRE LOTTE

### Case Reports

Metastasizing atrial myxoma: a case with multiple subcutaneous tumours 239
N.OKADA, T.YAMAMURA, Y.KITANO, T.NAKAMURA, H.KAMIDO, Y.MATSUZAWA, S.KATAYAMA AND K.OHARA
A syndrome of keratosis palmo-plantaris congenita, pes planus, onychogryphosis, periodontosis, arachnodactyly and a peculiar acro-osteolysis 243
J.M.PULIYEL AND K.S.SRIDHARAN IYER

### Correspondence

Degos' disease; JULIA A.NEWTON AND M.M.BLACK 249
Cyclosporin A and psoriasis; B.E.MONK 249
Elevated serum amylase in toxic epidermal necrolysis; H.TAGAMI AND K.IWATSUKI 250
Reply; F.M.TATNALL, H.J.DODD AND I.SARKANY 250
Anthralin inflammation: lack of effect of topical thiocarbamide drugs; Y.PARAMSOTHY, C.M.LAWRENCE AND S.SHUSTER 251
Haematological consequences of methotrexate therapy; H.BAKER 253
Amiodarone-induced cutaneous photosensitivity; J.BOYLE 253
Reply; J.FERGUSON AND B.E.JOHNSON 254
Arterial lesions of the lower lip; A.FRUMKIN 255
Reply; R.J.MARSHALL AND B.J.LEPPARD 255

**Book Reviews** 256

**News and Notices** 258

# British Journal of Dermatology

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# British Journal of Dermatology

## CIRD Symposium:
## Advances in Skin Pharmacology VI.
## Toxicity After Topical Application

4–6 October 1985, Sophia Antipolis, France

Edited by H.Schaefer, B.Shroot, M.Darmon, C.Hensby, W.Schalla and M.Prunieras

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# Foreword and Acknowledgements

The Sixth CIRD Symposium took place in Sophia Antipolis from 4 to 6 October 1985 and welcomed some 220 delegates from all over the world. The theme of toxicology after topical administration of drugs was selected because it presents for Skin Pharmacology one of the most important but poorly studied areas in the speciality.

Although such a meeting could not formulate official guidelines for cutaneous toxicology, it was constantly stressed that the cutaneous route of administration warranted special attention.

Much emphasis was placed on absorption and pharmacokinetics, so much so that the next Symposium will be devoted to this subject (see inside back cover).

We are indebted to the following for their stimulating participation as chairmen: M.W.Greaves, F.Kemper and N.Lowe.

In addition, we wish to thank the following members of the CIRD staff who were responsible for the discussion summaries: D.Asselineau, B.Bernard, M.Bouclier, P.Brun, M.Dange, C.Delescluse, J.Gazith, J.C.Jamoulle, A.Maloubier, S.Michel, M.Regnier, S.Robinson, M.Verschoore and O.Watts.

Finally, we express our very special gratitude to P.Reichert for the excellent organizational and secretarial work.

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*British Journal of Dermatology* (1986) **115**, Supplement 31, 1–8.

# Experimental evidence that skin carcinogenesis is a multistep phenomenon

F.MARKS AND G.FÜRSTENBERGER

German Cancer Research Center, Institute of Biochemistry, Im Neuenheimer Feld 280, 6900 Heidelberg,
Federal Republic of Germany

The process by which a tumour develops is complex. After contact with a carcinogenic agent it may take more than 20 years before the disease becomes detectable. From the experimental point of view, it is highly desirable to divide this process as far as possible into well defined stages, each of which can then be investigated separately. By repeating the experimental design which enabled Yamagiwa and Ichikawa (1914) to induce tumours in laboratory animals by means of a chemical agent (i.e. painting of rabbit skin with coal tar) for the first time, several investigators observed an acceleration of tumour development by *per se* non-carcinogenic manipulations such as wounding or treatment of skin with irritants. These observations prompted Friedwald and Rous (1944) to distinguish between two stages of skin carcinogenesis, which they called initiation and promotion. At about the same time, Mottram (1944), Berenblum (1941) and Berenblum and Shubik (1947) introduced a standardized experimental approach which is still in use. To achieve initiation they proposed treating mouse skin topically with a carcinogen in a 'subthreshold' dose, i.e. with a dose which is so small that the animals do not develop tumours during their lifetime (Mottram, 1944). For promotion, chronic application of the highly irritant croton oil, the seed oil of *Croton tiglium*, was carried out (Berenblum, 1941). Today, the pure active principles of croton oil, the phorbol esters and also related compounds derived from other natural sources are used as promoting agents (Fig. 1).

By employing this approach, it was found that initiation is a more or less irreversible process (Berenblum & Shubik, 1949), whereas promotion is quickly reversible, and that the initiation-promotion sequence of treatment could not be inverted (Berenblum & Haran, 1955; Fig. 2). From these observations, Berenblum drew the conclusion that promoters and initiators do not exhibit additive effects, but that promotion completes a process which was started with initiation.

Initiation can be achieved by many carcinogenic agents regardless of whether they are of chemical, physical or viral nature. The idea that initiation is due to an interaction of the agent with cellular DNA which results in a somatic mutation or 'genotoxic effect' has become a generally accepted concept. This theory is based on the following evidence: irreversibility of initiation, a far-reaching correlation of initiation and mutagenic efficacy of carcinogens, binding to or damage of cellular DNA, the monoclonality of the resulting tumours, and over-expression of a point-mutated H-ras-oncogen in skin tumours generated by the initiation-promotion approach (Balmain *et al.*, 1984; Balmain, personal communication). However, it should be kept

Correspondence: Dr F.Marks.

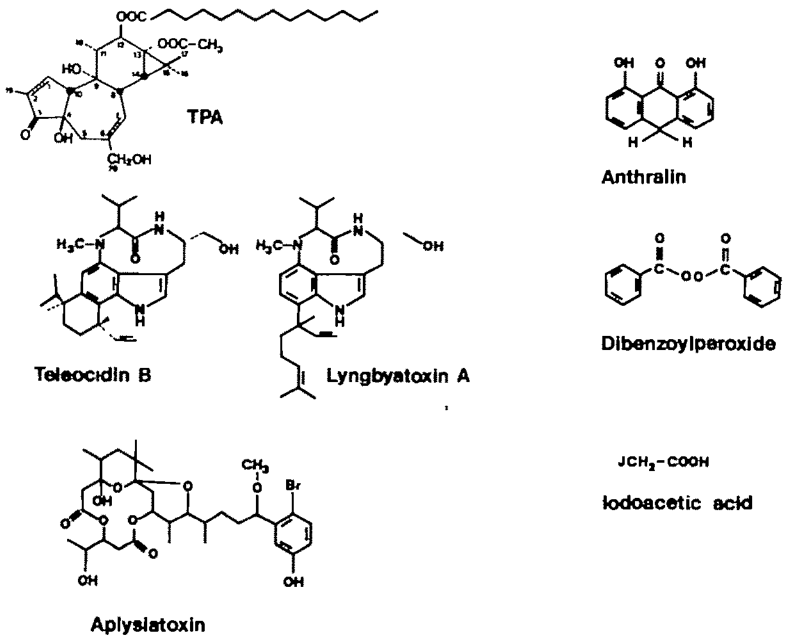

FIGURE 1. Skin tumour promoters. 12-o-Tetradecanoylphorbol-13-acetate (TPA), the active principle of croton oil, represents the phorbol ester family of promoting agents (Hecker, 1978). Teleocidin, lyngbyatoxin and aplysiatoxin are strong promoters isolated from marine organisms (Fujiki *et al.*, 1984). The other compounds are examples of relatively weak promoters. (From Marks & Fürstenberger, 1985).

in mind that this evidence is indirect and that there is as yet no means by which one can distinguish an initiated cell from a normal cell. The only way to prove initiation is to promote tumour development.

In the classical target organ of experimental two-stage tumorigenesis, the animal skin, promotion results in sustained epidermal hyperplasia which seems to be an essential condition for tumour development (Sisskin, Gray & Barrett, 1982). Provided that the promoter is applied at optimal dose and time schedule, the first tumours arise after 6 weeks of promoter treatment, and the full response is seen after about 12–15 weeks (Fig. 3). The great majority of these tumours are benign papillomas which do not exhibit growth autonomy but disappear with different decay rates when the treatment with the promoter is stopped (Burns *et al.*, 1978). Only 10–20% of these papillomas achieve proliferative autonomy, and 5–10% may eventually progress to malignancy. The progression rate does not depend on the promoter dose but can be increased by a second treatment with initiating agents (Hennings *et al.*, 1983). This observation has been interpreted to indicate that an additional genetic alteration is necessary for malignant progression.

As far as the nature of the initiated cell is concerned, several concepts have been put forward, all attempting to explain why an initiated cell remains silent and does not give rise to a tumour in the absence of promotion.

The simple assumption would be that promotion is due to nothing but permanent growth stimulation which is necessary to make the non-autonomously growing papillomas visible. Based on experiments made with epidermal cell cultures, Yuspa (1984) proposed an initiated

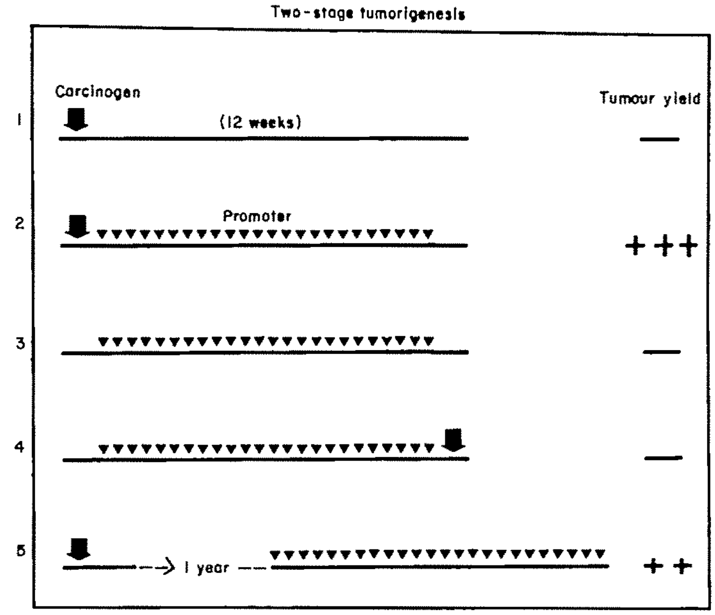

FIGURE 2. Scheme representing the classical 'Berenblum-Mottram-approach' of two-stage tumorigenesis in mouse skin. The scheme shows the essentials of the approach: (i) that neither a 'subthreshold' dose of a carcinogen (1) nor chronic application of a promoter (3), but only the combination of both treatments in a correct order (2, 4) results in tumour development; and (ii) that epidermis exhibits a 'memory' for initiation (5). For experimental details see Figure 3. (From Marks & Fürstenberger, 1985).

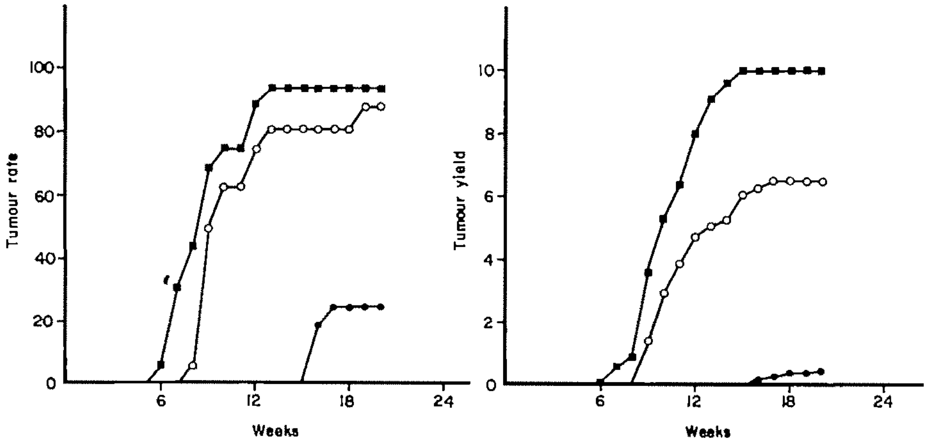

FIGURE 3. Typical outcome of an initiation-promotion experiment of tumorigenesis in mouse skin (strain NMRI). Initiation is carried out by a single total application of 100 nmoles 7,12-dimethylbenz[a] anthracene in 0·1 ml acetone at zero time. One week later promotion is started by a local application of TPA (in the doses indicated) in 0·1 ml acetone. The TPA treatment is repeated twice weekly over the whole time period of the experiment. Lefthand diagram: percentage of tumour-bearing animals in groups of 20 animals. Righthand diagram: average number of papillomas per animal. ● TPA 1 nmol; ○ TPA 5 nmol; ■ TPA 10 nmol; 0·2 nmol TPA: no tumours.

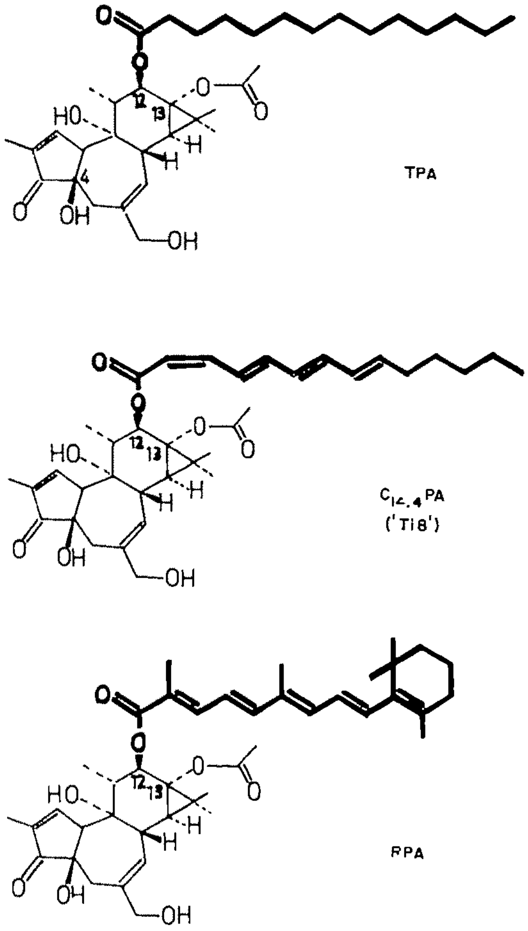

FIGURE 4. The 'disarmament' of the phorbol ester tumour promoter TPA. Upon introduction of conjugated double bonds into the $C_{14}$-acyl residue (yielding the natural product 'Ti8') or replacement of the $C_{14}$-acyl residue by the retinoyl residue (yielding the semisynthetic compound RPA), the ability of TPA to accelerate tumour development in the Berenblum-Mottram experiment (Fig. 2) is selectively abolished, whereby its irritant and hyperplasiogenic activities are retained.

cell to be a benign tumour cell which in the course of promotion is released from local growth restraints, thus being enabled to undergo clonal expansion. However, a concept such as this is unable to explain the observation that not every agent which induces epidermal hyperplasia is a promoter. To deal with this fact an additional observation was taken into account in the hypothesis, i.e. that a promoting agent not only stimulates cellular proliferation, but in addition accelerates the rate of terminal differentiation of epidermal cells. The combination of both growth stimulation and enhancement of terminal differentiation is thought to provide a selective advantage for tumour cells over the surrounding tissue.

Another, not necessarily conflicting, concept defines an initiated cell as a tumour cell, the neoplastic phenotype of which is still unexpressed (Boutwell, 1964). Even under permanent growth stimulation, such a cell would behave like a normal epidermal cell unless it underwent

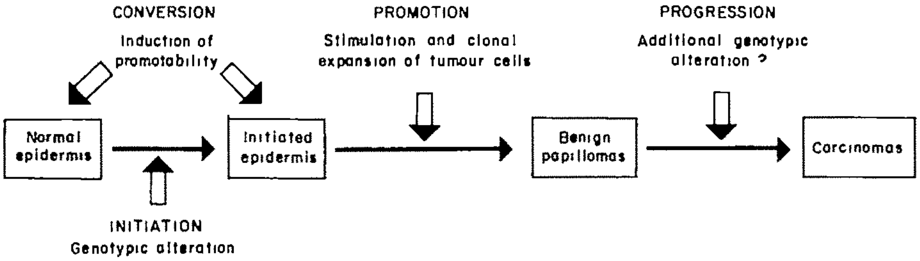

FIGURE 5. The four stages, initiation, conversion, promotion and malignant progression of experimental carcinogenesis in mouse skin. Initiation is carried out by a single local treatment with a carcinogenic agent, promotion by chronical application of a promoter such as the phorbol ester RPA, using the approach described for Fig. 3. For conversion, i.e. induction of promotability, a single topical application of a convertogenic agent (for instance TPA) or single skin wounding either before or after initiation is sufficient. Between conversion and promotion the experiment can be interrupted for several months (NMRI mice), whereas an increase of the time interval between two subsequent promoter treatments (from 3–6 days up to 2 weeks) results in a pronounced drop of tumour yield. The progression from the benign to the malignant state can be accelerated by an additional treatment of papilloma-bearing skin with a carcinogenic agent.

phenotypic expression. This would mean that promotion may consist of two stages, expression of the neoplastic phenotype, originally called 'conversion' of the initiated cell into a 'dormant' tumour cell, and growth stimulation, originally called 'propagation'. Not every mitogenic agent, but only such substances that additionally show the 'convertogenic effect', would be promoters. This concept was originally based on the finding that in initiated mouse skin the promoting effect of limited treatment with the phorbol ester TPA could be completed by subsequent chronic application of the non-promoting mitogen turpentine (Boutwell, 1964). Later, similar experiments were carried out with a diterpene ester, mezerein, as a 'propagating' agent (Slaga *et al.*, 1980).

Perhaps the most convincing evidence that promotion involves more than just permanent growth stimulation is provided by the experimental 'disarmament' of phorbol ester tumour promoters (Fig. 4). This approach emerged from the observation that a phorbol ester derived from *Euphorbia tirucalli* (Fürstenberger, 1976; Fürstenberger & Hecker, 1986), which differed from TPA by the presence of four conjugated double bonds located in the tetradecanoyl residue of the molecule, was indeed a strong hyperplasiogenic skin irritant but only a very weak tumour promotor (Fürstenberger, 1976; Marks, Bertsch & Fürstenberger, 1979). An even more efficient way to abolish tumour promoting efficacy is to replace the tetradecanoyl side chain of TPA by the five-fold unsaturated retinoyl residue (Fig. 4; Fürstenberger *et al.*, 1981).

The resulting 12-o-retinolyphorbol-13-acetate (RPA) exhibits practically the same irritant and hyperplasiogenic effects as TPA, including the induction of special biochemical events characteristic of the hyperplastic response, but is almost unable to evoke tumour growth in initiated mouse skin of the NMRI-strain. On the other hand, RPA turned out to be an efficient and suitable 'stage II promoter', in that it can complete the promoting effect of one single TPA application (stage I of promotion or 'conversion') when subsequently applied over a period of several weeks.

This two-stage protocol of tumour promotion has provided new insights into the events involved in multistage skin carcinogenesis. Since the result of the experiment appears to be independent of whether stage I treatment is carried out before or after initiation (Fürstenberger *et al.*, 1985), conversion was suggested to be defined as a discrete step of multistage tumorigenesis rather than as a stage of promotion, bearing in mind that promotion is defined strictly as a process which has to follow initiation (Fig. 5). In other words, promotion would then be synonymous with what was formerly called 'propagation' or 'stage II', and RPA would be defined as a promoting agent, whereas TPA represents a group of agents which, in addition to promoting effeciency, exhibit convertogenic potency.

The operational definition of conversion is that in this stage the tissue is made sensitive for the effects of a promoter, i.e. epidermis is converted into a state of increased promotability. Whereas promotion can most probably be explained by clonal expansion of tumour cells due to a selection process occurring in the course of sustained epidermal hyperplasia, the mechanism of conversion is still a mystery. The observation that conversion can be carried out prior to initiation is only difficult to reconcile with the concept that a convertogenic agent induces the expression of the neoplastic phenotype. On the other hand, in the course of conversion, a situation which allows spontaneous phenotypic expression during subsequent initiation may be created in the tissue. It is noteworthy that in contrast to promotion which is quickly reversible, the state of promotability induced by conversion dies away slowly with a half-life of 10–12 weeks in NMRI mice (Fürstenberger *et al.*, 1985). In addition, conversion depends on DNA synthesis (Kinzel *et al.*, 1984) and is suppressed by inhibitors of the arachidonic acid metabolism and by retinoic acid (Marks & Fürstenberger, 1984). As yet, no evidence can be provided for involvement of protein kinase C, the 'phorbol ester receptor', and superoxide anion radicals in conversion, although both are thought to play a key role in promotion (reviewed by Ashendel, 1985; Marks & Fürstenberger, 1985; Troll & Wiesner, 1985).

Conversion can also be induced by skin wounding (Fürstenberger & Marks, 1983). Speculation has arisen, therefore, that conversion could be due to the induction of special proliferative and/or metaplastic processes thought to be involved in the wound response (Marks *et al.*, 1982). Such processes would either activate existing initiated cells or convert the tissue into a state in which subsequent initiation results immediately in promotable tumour cells. It is highly probable that such processes are controlled by endogeneous factors or 'wound hormones', and that the effects of such factors are mimicked by convertogenic agents such as TPA.

Thus, a fairly complex picture of processes involved in experimental skin carcinogenesis has emerged. It should be emphasized, however, that the stages of skin carcinogenesis are defined operationally on the basis of distinct experimental designs with certain mouse strains. As yet, it is not certain whether the knowledge obtained from such experiments can be generalized as far as tumour development in other organs, in other animal species or in humans is concerned.

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## DISCUSSION SUMMARY

Several points were discussed:

(1) It is known that initiators interact with DNA, whereas convertogenic agents (such as

TPA) do not. Pure convertor has yet to be isolated. TPA allows conversion, but also promotion. TPA binds to protein kinase C, but also to other binding sites which are not very well defined. The binding of TPA to protein kinase C is very short lived because of the high clearance in the tissue and because signal transmitting systems become refractory. However, there is indirect evidence that the protein kinase C is probably not involved in conversion.

(2) Recently, a specific activator of protein kinase C in the liver has been tested. The study of its effect on epidermis has yet to be done. This would allow one to ascertain whether activation of protein kinase C plays a role in hyperplasia.

(3) It is possible to imagine that stem cells are the target for initiation, and amplifying cells the target for conversion. However, it remains to be explained why TPA acts specifically on long-retaining labelling cells and why RPA acts on all epidermal cells.

(4) Prostaglandins of the two series are involved in promotion. On the other hand, it is possible to convert prostaglandins of the series two into the series three by feeding mice with EPA. It would be interesting to investigate the effect of TPA in the latter case.

(5) There are species differences in terms of promotion. Several explanations for this have been given; refractoriness to chronic hyperplasia could be one of them.

(6) Amongst the anti-inflammatory agents, the non-steroidal anti-inflammatories are potent inhibitors of ODC, DNA synthesis and promotion.

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# Pharmacological modification of epidermal detoxification systems

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## SUMMARY

The skin is a major interface between the body and the environment and possesses membrane-bound cytochrome P-450-dependent enzyme activity that is capable of both detoxifying endogenous and exogenous substrates and enhancing the toxic effects of selected substances.

The use of chemical inhibitors of enzyme activation by P-450-dependent enzymes represents one possible approach to controlling toxic responses in target tissue such as the skin. Our data indicate that certain polyphenols and imidazoles are potent inhibitors of epidermal carcinogen metabolism, and of the DNA binding and the carcinogenicity of PAHs such as BP. The use of such agents may offer a novel approach to the prevention of environmentally induced cancer.

The skin is a major interface between the body and its environment and as such is particularly vulnerable to injury by a variety of exogenous physical and chemical agents. Despite this apparent vulnerability, the skin possesses a complex structure that constitutes a formidable barrier against the penetration of many environmental toxins. In the past, many believed that the skin was little more than a passive metabolically inert barrier, but dermatological research in the past several decades has shown unequivocally that cutaneous tissue is a major site of multiple forms of metabolic activity (Bickers, 1980). The consequences of the type of metabolic activity that is dependent upon the heme-protein cytochrome P-450 are not fully understood as yet. However, it is known that this enzyme system can both detoxify potentially noxious drugs and chemicals as well as convert relatively inert precursor substrates into highly reactive metabolites that can bind avidly to cellular macromolecules, thereby producing toxic injury.

This xenobiotic metabolizing enzyme system is membrane-bound, requires NADPH and molecular oxygen for catalytic activity, and has as its terminal oxidase the heme-protein, cytochrome P-450. This enzyme system, also known as the mixed function oxidase or mono-oxygenase, has been most carefully studied in mammalian liver where it has been solubilized, purified to virtual homogeneity, and its amino acid sequence determined (Guengerich, 1979; Mizukami *et al.*, 1983). It is apparent that multiple forms of hepatic cytochrome P-450 exist, and that these isozymes differ in terms of their substrate specificity and in their response to the administration of various drugs and xenobiotics. In summary, it is known that P-450-dependent mono-oxygenases are inducible enzymes whose synthesis is increased following exposure to certain xenobiotics. This results in generally increased rates of metabolic transformation of appropriate substrates.

While much important knowledge has been gained from studies of hepatic P-450, there is much less information available regarding this enzyme system in extrahepatic tissues such as the skin. Since the skin is a major site of exposure to environmental agents, and since from a therapeutic viewpoint many drugs are applied directly to this tissue, it is essential that a more complete understanding of these metabolic events and their consequences be developed in cutaneous tissue. Furthermore, it is increasingly clear that P-450-dependent metabolic activity may play an important role in the generation of endogenous and exogenous substances with enhanced biological activity, and that modulation of such activity may offer an exciting new approach for diminishing toxic responses in cutaneous tissue.

## EPIDERMAL CYTOCHROME P-450

Several years ago, we showed that neonatal Sprague-Dawley rats are a useful animal model for the study of epidermal cytochrome P-450, and that the major proportion of this heme-protein is localized in this skin compartment or in organelles derived embryologically therefrom (Bickers, Dutta-Choudhury & Mukhtar, 1982; Mukhtar & Bickers, 1981).

A major drawback to measurement of epidermal cytochrome P-450 is the lability of the microsomal enzyme system *in vitro*. It was therefore necessary to develop special methodological approaches using antioxidants and glycerol to maximize the yield of spectrally detectable epidermal P-450.

A typical carbon monoxide (CO) difference spectrum for liver microsomal P-450 demonstrates a peak absorbance at 450 nm. In epidermal microsomes prepared from newborn animals, there is a peak absorbance under these conditions at approximately 453 nm. Microsomes prepared from animals pre-treated 24 h before sacrifice with a single topical application of the polyaromatic hydrocarbon carcinogen, 3-methylcholanthrene (3-MC), demonstrate increased amounts of cytochrome P-450 and a shift in the peak absorbance of the heme-protein to 451 nm (Bickers *et al.*, 1982). This is consistent with studies in liver and other tissues which have shown that certain carcinogenic polyaromatic hydrocarbons induce an isozyme of cytochrome P-450 that demonstrates a 2 nm shift to the blue in peak absorbance. These findings indicate that neonatal Sprague-Dawley rats possess a highly inducible microsomal P-450 system, and that this strain represents an excellent animal model for the study of xenobiotic metabolism in the skin.

In recent studies designed to characterize the nature of the 3-MC inducible epidermal P-450 more fully, we have employed a new approach for measuring epidermal cytochrome P-450 using an anion exchange high performance liquid chromatography (HPLC) system. Epidermal microsomes were prepared under conditions that minimize oxidative injury to this lipid-rich membranous component, and were solubilized with sodium cholate. The solubilized preparation was injected onto the HPLC and absorbance of the heme-protein measured at 405 nm. Solubilized microsomes from control animals demonstrated a single small peak that appears to be the uninduced basal heme-protein. Solubilized microsomes prepared from animals pre-treated with topically applied 3-MC show several new peaks analogous to isozymes of P-450 found in liver microsomes of 3-MC treated animals (Kotake & Funae, 1980). These findings indicate that mammalian epidermis possesses multiple isozymes of cytochrome P-450 analogous to those present in the liver, although present in smaller amounts. It is therefore likely that the skin is capable of metabolizing a broader range of both endogenous and exogenous substrates than previously suspected.

## HUMAN EPIDERMAL CYTOCHROME P-450-DEPENDENT XENOBIOTIC METABOLISM

The evidence indicating that epidermis is an active site of extrahepatic cytochrome P-450-dependent enzyme activity in rat skin prompted a search for this type of catalytic activity in human epidermis. These studies were carried out in collaboration with Drs Cynthia Marcelo and John Voorhees at the University of Michigan (Bickers *et al.*, 1984). Freshly keratomed human epidermis was obtained from both normal volunteers and from visibly uninvolved skin of patients with psoriasis. The tissue was divided immediately into two segments, one of which was added to tissue culture medium containing a small amount (10 $\mu$M) of the polyaromatic hydrocarbon benz[a]anthracene (BA). Control medium was treated with solvent (acetone). The epidermal segments were incubated in this short-term organ culture system for 24 h, harvested and washed, and microsome-enriched fractions prepared for measurement of the cytochrome P-450-dependent enzyme known as aryl hydrocarbon hydroxylase (AHH). This enzyme functions in the transformation of polycyclic hydrocarbons like benzo[a]pyrene (BP) into a spectrum of metabolites including phenols, quinones, and dihydrodiols. Phenolic metabolites can then be quantitated readily by a fluorescence assay. There was detectable epidermal AHH activity in untreated samples from both normal and psoriatic individuals. Furthermore, incubation of the samples with BA caused a 1·5–4-fold induction of the human epidermal enzyme. It is of interest that inducibility of the enzyme was found to be greater in psoriatic than in normal epidermis, although the relationship, if any, of this observation to the pathogenesis of the disease remains unknown (Bickers *et al.*, 1984).

In further studies, it was shown that human epidermal AHH activity is linear with respect to time of incubation, to protein concentration, and to substrate concentration, and that the pH maximum is 7·4. Furthermore, the Km of the enzyme for BP was found to be 0·62 $\mu$M. Since the levels of P-450 in human epidermis are insufficient for measurement of a CO-difference spectrum, it is necessary to use indirect methods to establish the presence of this heme-protein. The human epidermal enzyme was shown to be inhibited by 7,8-benzoflavone, a known inhibitor of P-450-dependent enzymes in other tissues; it was also shown to require both NADPH and oxygen for catalytic activity and CO-inhibited enzyme activity. These findings are consistent with the observation that human epidermal AHH is a cytochrome P-450-dependent enzyme.

## EPIDERMAL AHH, CARCINOGEN METABOLISM AND CANCER INDUCTION

PAHs are ubiquitous environmental pollutants which are generated during the incomplete combustion of organic substances (Guerin, 1978). Many of the PAHs are now known to have cytotoxic, mutagenic and carcinogenic effects in experimental animals and in humans (Gelboin, 1980). Several of these compounds cause skin cancer both in experimental animals, and in certain circumstances, in humans (Henry, 1947). PAHs can elicit malignant transformation of a variety of cell types in culture (Gelboin, 1980; Baird & Diamond, 1978; Conney, 1982). The PAHs themselves are relatively inert compounds and essentially act as precarcinogens that must first undergo metabolic activation to biologically reactive metabolites that are the actual oncogenic agents (Grover, 1979; Sims & Grover, 1974). The ultimate carcinogenic metabolites of certain PAHs are highly reactive bay-region diol-epoxides that can bind covalently to cellular macromolecules including RNA, DNA and proteins (Jerina *et al.*, 1977; Sims, 1980). It is generally agreed that the covalent interaction of reactive diol-epoxides of certain PAHs with macromolecules such as DNA represents an essential step in tumour induction (Jerina *et al.*, 1977; Sims, 1980). Of the PAHs, BP has been employed widely as a prototype for metabolic studies, and DMBA for skin carcinogenesis studies. It is known that BP is metabolized in numerous tissues to a wide variety of organic solvent-soluble metabolites, including epoxides, dihydrodiols, quinones and phenols, in addition to water-soluble glutathione, glucuronide and sulphate conjugates (Gelboin, 1980). Studies over the past decade have identified one of the isomeric metabolites of BP known as BP-7,8-diol-9,10-epoxide I as the ultimate carcinogenic metabolite of this hydrocarbon (Conney, 1982). The formation of this metabolite is catalysed by three successive steps: first, production of BaP-epoxide by AHH, a cytochrome P-450-dependent mono-oxygenase; second, conversion of the epoxide to a dihydrodiol by epoxide hydrolase, and third, metabolism of the dihydrodiol to a diol-epoxide by AHH (Conney, 1982; Bend *et al.*, 1982). The reactive epoxide intermediates may be detoxified by conjugation with glucuronide or sulphate (Bend *et al.*, 1982), or be activated further to electrophilic moities which can undergo covalent binding to DNA (Grover, 1979; Conney, 1982). These enzymatic pathways for the metabolic activation and inactivation of BP are known to occur in both hepatic and extrahepatic tissues, including skin (Bickers *et al.*, 1982).

As a corollary to these observations regarding the relationship between AHH activity and malignant transformation, it can be stated that the extent of the activity *and* inducibility of AHH in a target tissue exposed to carcinogens such as BP will be a major determinant of cancer risk in that tissue.

## ANTICARCINOGENS AS AN APPROACH TO THE PREVENTION OF CUTANEOUS MALIGNANCY

Knowledge that P-450-dependent mono-oxygenases play a major role in the metabolic activation and the carcinogenicity of PAHs such as BP has stimulated interest in identifying inhibitors of enzymes that participate in the generation of the reactive oncogenic metabolites. As discussed above, there are multiple enzymatic steps in the metabolic activation of carcinogens like BP that could be potential targets for this approach.

Phenolic compounds such as ellagic acid, ferrulic acid, caffeic acid, and chlorogenic acid are widely distributed in the plant kingdom (Sondheimer, 1964). The studies of Wood *et al.* (1983) showed that ellagic acid, ferrulic acid, chlorogenic acid and caffeic acid have substantial anti-mutagenic activity towards the bay region diol-epoxides of selected PAHs. Of these four plant phenols, ellagic acid was shown to be exceptionally potent in this regard. Earlier studies (Wattenberg, Coccia & Lam, 1980) had shown a reduction of about 40% in BP-induced neoplasia of forestomach in mice fed diets containing 1·1% caffeic acid or ferrulic acid. Dietary caffeic acid causes a reduction of almost 50% in DMBA-induced genotoxicity, as expressed by the formation of micronuclei in bone marrow of mice (Newmark, 1984). Furthermore, chlorogenic acid is a potent inhibitor of mutagenesis caused by a nitrosation product of methylurea (Stich, Rosin & Bryson, 1982).

Other phenolic compounds such as gallic acid, tannic acid, myricetin, anthraflavic acid, monolactone, alizarin and purpurogallin are generally known as flavonoids and are present in a variety of edible fruits and vegetables (Griesebach, 1979; Brown, 1980). Many of these plant phenols are ingested in the human diet in amounts as high as 1 g/day (Brown, 1980). The studies of Stich *et al.* (1982) showed that tannic acid inhibits the mutagenesis of methylurea by inhibiting the formation of nitrosation products. Buening *et al.* (1981) observed that the addition of the polyhydroxylated flavonoids, apigenin, chrysin, fisetin, myricetin, and kaempferol, to a human liver microsomal system significantly inhibited the metabolism of BP.

Furthermore, quercetin, morin, and kaempferol are known to be potent inhibitors of NADPH cytochrome P-450 reductase, the enzyme that provides reducing equivalents for cytochrome P-450 which is essential for the catalytic activity of the latter (Buening *et al.*, 1981). Besides their inhibitory effects on BP metabolism, Huang *et al.* (1983, 1985) have shown recently that certain flavonoids inhibit the mutagenicity of $(\pm)7\beta,8\alpha$-dihydroxy-$9\alpha$-$10\alpha$-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene [(±)BPDE], the major ultimate mutagenic and carcinogenic metabolite of BP. Myricetin and tannic acid were especially effective in this regard, whereas flavonoids without hydroxyl groups or with methylated hydroxyl groups were ineffective (Huang *et al.*, 1983, 1985). These investigators suggested that the anti-mutagenic activity of hydroxylated flavonoids was apparently due to their direct interaction with the diol-epoxide, since myricetin, robinetin, quercetin, tannic acid and anthraflavic acid accelerated the disappearance of the mutagen from dioxane-water solutions at rates that correlated with their ability to inhibit mutagenicity. These observations prompted us to explore the possibility that plant polyphenols could have anti-carcinogenic effects in the skin.

Our studies have shown that ellagic acid is a potent inhibitor of epidermal microsomal AHH activity and of enzyme-mediated binding of BP to both calf thymus DNA *in vitro* and to epidermal DNA *in vivo* (Mukhtar *et al.*, 1984a). Ellagic acid has also been shown to inhibit the covalent binding to BP to mouse epidermal DNA in organ culture (Shugart & Kao, 1984). Dixit *et al.* (1985) have shown that ellagic acid inhibits the metabolic activation and the binding of BP and BP-7,8-diol to DNA in cultured mouse lung. In further studies, we have shown that ellagic acid is a potent inhibitor of BP metabolism and its subsequent glucuronidation, sulphation and covalent binding to DNA in cultured Balb/c mouse keratinocytes (Mukhtar *et al.*, 1984b). Furthermore, we have demonstrated that topical application of ellagic acid to the skin of Balb/c mice results in substantial protection against 3-MC-induced skin carcinogenesis by delaying the latent period prior to tumour development (Mukhtar *et al.*, 1984c). This finding has been confirmed by Lesca (1983).

Chronic oral feeding of ellagic acid in drinking water (0·3 mg/l for 16 weeks) or acute intraperitoneal administration (50 mg/kg for 5 consecutive days) of the compound to Balb/c mice resulted in 20–25% decreases in hepatic and pulmonary cytochrome P-450 levels (Das, Bickers & Mukhtar, 1985). Hepatic and pulmonary AHH and 7-ethoxycoumarin O-deethylase activities in both groups of ellagic acid-treated animals were 33–52% and 38–43% lower than their respective non-ellagic acid-treated controls. Hepatic as well as pulmonary aminopyrine N-demethylase and epoxide hydrolase activities were unchanged in both groups of the ellagic acid-treated mice. Hepatic glutathione S-transferase activity towards BP-4,5-oxide or 1-chloro-2,4-dinitrobenzene as substrates was found to be enhanced 51–79% and 38–58% in both groups of animals. HPLC analysis of organic solvent soluble metabolites of BP by liver and lung microsomes indicated a substantial inhibition of diol formation (including BP-7,8-diol), as well as phenols and quinones. In liver, these inhibitory effects were more pronounced after oral feeding than after intraperitoneal administration. Our results indicate that both acute and chronic administration of ellagic acid inhibits the metabolic activation of BP and/or enhances glutathione S-transferase activity. Thus, modulation of PAH metabolism by ellagic acid may be related to the anti-carcinogenic effects of this compound in murine skin.

In further studies, we attempted to verify that chronic oral feeding of ellagic acid in drinking water has anti-carcinogenic effects in the skin. Balb/c mice ingested ellagic acid in drinking water (average intake 18 μg/animal/day) for 120 days.

AHH, 7-ethoxycoumarin O-deethylase and epoxide hydrolase activities in skin and liver of ellagic acid-fed mice were significantly lower than those in control animals. The extent of binding of topically applied $^3$H-BP to epidermal DNA was 54% lower in the ellagic acid-fed mice as compared with control animals. In further studies, the incidence of skin tumours in groups of animals receiving topically applied 3-MC (1·5 μmol twice weekly for 16 weeks) was evaluated. The animals pre-treated with orally administered ellagic acid had significantly fewer skin tumours, and the latent period prior to the onset of tumours was substantially prolonged. These studies indicate that chronic oral feeding of low doses of ellagic acid to mice affords substantial protection against 3-MC-induced skin carcinogenicity, and that it is likely that the mechanism for this anti-carcinogenic effect relates to inhibition of metabolic activation of the carcinogen.

A number of investigators have shown that imidazole-containing compounds, including certain widely used drugs like cimetidine and benzimidazole, and antifungal agents such as ketoconazole and clotrimazole, can alter hepatic xenobiotic metabolism by inhibiting microsomal cytochrome P-450-dependent mono-oxygenase activities (Sheets & Mason, 1984; Kahl *et al.*, 1980). These observations led us to assess the effect of imidazole compounds on carcinogen metabolism in the epidermis.

We have shown that clotrimazole is a potent inhibitor of the epidermal metabolism of BP and its microsomal enzyme-mediated binding both to neonatal rat epidermal DNA *in vivo* and to calf thymus DNA *in vitro* (Mukhtar *et al.*, 1984d). Varying concentrations of clotrimazole added to *in vitro* incubation systems resulted in a dose-dependent inhibition of cytochrome P-450-dependent microsomal AHH in control animals, as well as in animals pre-treated with topical application of known inducers of the enzyme. Inhibition of epidermal AHH by topically applied clotrimazole was time- and dose-dependent. The 50% inhibition of clotrimazole for epidermal AHH ranged from 0·12 to 0·25 μM, which suggests that clotrimazole is among the most potent inhibitors of epidermal AHH yet identified. Clotrimazole was also found to be a potent inhibitor of epoxide hydrolase activity *in vitro* with 50% inhibition at a concentration of 0·1 mM. High-pressure liquid chromatographic analysis of the metabolism of BP in rat epidermal microsomes revealed substantial inhibition of BP metabolism prepared from untreated animals, as well as animals pre-treated with inducers of the enzyme. Furthermore, a single topical application of clotrimazole resulted in 80% and 30% induction of epidermal and hepatic glutathione S-transferase activity, respectively. Topical application of clotrimazole to the skin of Balb/c mice substantially increased the latent period for the development of skin tumours by 3-MC.

These studies indicate the clotrimazole is an extremely potent inhibitor of epidermal BP metabolism and of the DNA-binding of carcinogenic PAHs and also enhances the activities of enzymes necessary for detoxification of such PAHs. Clotrimazole also reduces the formation of carcinogenic and mutagenic metabolites of BP *in vitro*, and inhibits induction of skin tumours by the PAH. These data indicate that imidazoles such as clotrimazole offer promise as agents for reducing the risk of cutaneous cancer in skin exposed to carcinogenic PAHs.

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## DISCUSSION SUMMARY

Dr Bickers made the following comments:

(1) Cytochrome P-450 can sometimes be induced by UVB irradiation, but it is unclear whether this is some form of toxic injury to keratinocytes and an activation process independent of enzyme induction, or a stimulus of the synthesis of new heme-protein analogous to that produced by chemicals. Using inhibitors of protein synthesis, he could not abolish the UV effects of P-450. This suggests that UVB light may be having some toxic effect that allows more material to be measured spectrally, or enhances the availability of substrate for the enzyme under *in vitro* conditions.

(2) In the studies with dietary ellagic acid (E.A.), the compound was administered orally throughout the whole course of the experiment. Topically applied clotrimazole was applied 2–3 h prior to each topical application of the carcinogen.

(3) The variety of effects of E.A. on living systems were not presented here.

(4) It is estimated by nutritionists that the average human ingests normally approximately 1 g of plant phenols E.A. in a diet on a daily basis in one form or another. It is not known whether this dosage has an anti-carcinogenic effect, and the influence of E.A. on the metabolic pathways also remains an unanswered question.

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# Defined culture systems for pharmacological and toxicological studies

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Cell culture systems appropriate for skin pharmacology studies have been reviewed in this journal recently (Prunieras & Delescluse, 1984). Emphasis at that time was placed on keratinocyte culture systems, reflecting both substantial recent progress in this field and the greater relevance of this cell type, relative to the previously utilized dermal fibroblasts to manifestations of skin disease and dermatological therapy. The goal of this present paper is to summarize, update and expand this earlier report, and to organize in a comparable manner data on culture systems for the human epidermal melanocyte, a second cell type commonly affected in the pathogenesis and therapy of cutaneous disease.

## KERATINOCYTE CULTURE

Human epidermal keratinocyte culture* was advanced markedly by Rheinwald and Green (1975), who demonstrated that serial cultivation of highly purified cells from newborn and adult donors could be achieved using a lethally-irradiated 3T3 fibroblast 'feeder layer' and serum-supplemented medium. Keratinocyte proliferative capacity and culture lifespan were subsequently improved in this system by addition of epidermal growth factor (Rheinwald & Green, 1977), and agents such as cholera toxin that stimulate cyclic AMP (Green, 1978). Other investigators have avoided the sometimes confounding presence of a second, fibroblastic cell type in this system by coating culture dishes with substrate materials such as fibronectin or collagen, and by markedly increasing keratinocyte seed density (Lui & Karasek, 1978; Gilchrest, Nemore & Maciag, 1980; Milo, Ackerman & Noyes, 1980; Peehl & Ham, 1980). More recently, refined use of hormone-supplemented media has permitted elimination of serum from keratinocyte culture systems (Maciag *et al.*, 1981; Tsao, Walthall & Ham, 1982; Boyce & Ham, 1983). Both the flexibility and the limitations of such systems for evaluation of pharmacological agents are discussed in the earlier review (Prunieras & Delescluse, 1984).

Elimination of serum from cell cultures has numerous advantages. First, serum lots are notoriously variable in their mitogenicity, and use of serum doubtlessly compounds the ever-present problem of repoducibility in keratinocyte studies. Second, unidentified components of serum may undermine experimental design, as was the case, for example, in some early experiments addressing the roles of vitamin A and fibronectin in the epidermis. In the absence of other safeguards, serum may also contribute to fibroblast contamination of keratinocyte cultures. Finally, serum provides such powerful and indiscriminate mitogenic stimulation that it may mask the contribution of more subtle but more physiological regulators of keratinocyte growth and differentiation.

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* The extensive literature regarding murine keratinocytes will not be reviewed here.

The serum-free system developed in our laboratory has thus far permitted relative quantitation of known or suspected keratinocyte mitogens (Gilchrest, Calhoun & Maciag, 1982), exploration of possible autocrine and paracrine growth regulation within the skin (Gilchrest *et al.*, 1983), partial characterization of a novel keratinocyte mitogen (Gilchrest *et al.*, 1984b), detection of age-associated losses in proliferative capacity even within adulthood (Praeger & Gilchrest, 1985), and identification of a candidate for an epidermal chalone (Yaar *et al.*, 1985). The sensitivity and reproducibility of this and similar systems holds great promise for studies of drug effects on healthy and diseased skin.

Two modifications of the basic approach to keratinocyte cultivation deserve special mention because of the opportunities they provide for pharmacological investigation. So-called 'low' calcium ($Ca^{++}$) or $Ca^{++}$-depleted media, containing $<0{\cdot}1$ mM $Ca^{++}$ rather than the conventional 1·2–1·8 mM $Ca^{++}$, were first reported in murine keratinocyte systems (Hennings *et al.*, 1980) to yield monolayer cultures of apparently uniform, non-terminally differentiated cells, in contrast to the stratified differentiating colonies observed at higher $Ca^{++}$ concentrations. Human keratinocytes also respond to reduced $Ca^{++}$ with differentiation arrest, greatly enhanced growth rate and prolonged culture lifespan (Boyce & Ham, 1983), although in some systems comparable growth is achieved at 'physiological' $Ca^{++}$ levels (Gilchrest *et al.*, 1984b). This increased proliferation is particularly critical in serum-free systems and facilitates studies utilizing necessarily small human biopsy specimens. Further, the relative homogeneity of the cultured cell population may facilitate design and interpretation of experiments involving drug action, even though it has been elegantly demonstrated that the keratinocyte monolayer observed in $Ca^{++}$-depleted medium contains cells at different stages of differentiation (Watt & Green, 1982). These two properties of low $Ca^{++}$ systems have potential pharmacological usefulness independent of the system's physiological relevance, which is currently the focus of some debate.

The second modification is the use of air-liquid interface cultures in which colonies are maintained on collagen gels or other supports at the surface of the nutrient medium (Prunieras, Regnier & Woodley, 1983) to achieve complete epidermal differentiation with formation of a stratum corneum, in contrast to conventional 'submerged' cultures in which differentiation appears to be blocked at or below the stratum granulosum. The mechanism by which this physical arrangement alters keratinocyte behaviour *in vitro* remains to be determined. Nevertheless, the approach appears to provide for the present a valuable opportunity for studies of stratum corneum formation, ichthyotic disorders, and drugs affecting desquamation.

## MELANOCYTE CULTURE

Successful culture of human melanocytes was first reported nearly 30 years ago, but early systems allowed only for short-term growth of melanocytes as a minor cell component with a mitotic disadvantage relative to competing keratinocytes and dermal fibroblasts (Hu *et al.*, 1957; Cruickshank, Copper & Hopper, 1960; Cohen & Szabo, 1968; Kitano, 1970; Klaus, 1980). Eisinger and Marko (1982) first reported extensive proliferation of melanocytes from human neonates over several months without overgrowth by other cutaneous cell types in medium containing the tumour promoter phorbol 12-myristate-13-acetate (TPA), cholera toxin, and 5% fetal bovine serum, and attributed their success to the toxicity of phorbol ester for keratinocytes. More recently, Tsuji & Karasek (1983) noted preferential survival of melanocytes derived from both newborn and adult epidermis for up to 6 weeks as primary cultures in medium containing 10% calf serum, cholera toxin, a second agent to elevate cAMP, and 5-fluorouracil, an antimetabolite with greater toxicity for keratinocytes than for melanocytes in this system. Highly enriched long-term human melanocyte cultures were achieved by Nielsen & Don (1984) using a fibroblast-conditioned medium supplemented with 15% horse serum and polyamines to discourage keratinocyte overgrowth, after initial selection for melanocytes by discontinuous Percoll gradient centrifugation and a differential plating technique for the resulting cell suspension.

An alternative approach, developed in our laboratory, utilized the greater substrate dependency of cultured keratinocytes to obtain preferential melanocyte attachment and growth in a hormone-supplemented medium containing a potent, previously undescribed melanocyte growth factor extracted from bovine hypothalamus (Wilkins *et al.*, 1982). Newborn melanocytes underwent 16 cumulative population doublings (PD) routinely over 2–3 months, but the relatively low plating density required to avoid initiation of keratinocyte colonies was also disadvantageous to melanocytes, reduced their final yield, and virtually prohibited their cultivation from adult skin specimens. Subsequently, the system was modified to remove the problem of keratinocyte contamination while supporting good melanocyte proliferation and pigment production in the absence of tumour promotors such as TPA or chemotherapeutic agents with minimal serum supplementation (Gilchrest *et al.*, 1984a; Wilkins *et al.*, 1985).

In this modified system presently employed in our laboratory, disaggregated epidermal cell suspensions are plated on uncoated tissue culture dishes in a nutrient medium supplemented with 10 ng/ml epidermal growth factor, $10^{-9}$ M triiodothyronine, 10 $\mu$g/ml transferrin, 10 $\mu$g/ml insulin, $10^{-9}$ M cholera toxin, and the bovine hypothalamic extract as described above but dialysed against Tris-HCl buffer using 6000–8000 dalton dialysis tubing to yield a dialysed extract lacking the low molecular weight keratinocyte growth factor present in the crude extract (Gilchrest *et al.*, 1984b). Fetal bovine serum (FBS) is added at $\leq 2$ mg/ml (2%) to cultures at the time of inoculation and at the time of each passage, but is usually omitted from the medium after these initial feedings. These cultures, estimated to contain 2–4% melanocytes initially, consist for the first several days *in vitro* of large keratinocyte colonies with occasional interspersed dendritic cells. By the third week, the keratinocyte colonies persist only as refractile debris, due to lack of keratinocyte growth factor necessary for their survival in this culture system (Gilchrest *et al.*, 1984b). By the fourth week, cultures consist exclusively of melanocytes, as confirmed by both phase and electron microscopic examination (Fig. 1a). Melanocytes in such cultures can be serially passaged through approximately 10–14 post-primary population doublings (PD) when derived from newborn foreskin specimens and 4–8 PD when derived from adult truncal skin specimens. One to two PD per week are usually obtained. Addition of 2% FBS increases growth rate and culture lifespan substantially, but at the risk of eventual fibroblast contamination.

By comparison, in the other culture system for which growth kinetics are described (Eisinger & Marko, 1982), melanocytes derived from newborn foreskin or adult surgical specimens undergo approximately 1–1·5 PD or 0·25–0·5 PD per week respectively in the presence of 5% FBS, cholera toxin, and TPA, while essentially no growth is obtained in the absence of these combined supplements. More recently, these authors have found that various tissue extracts can be substituted for TPA in this system in the presence of 10% FBS to yield comparable growth rates, and that the most stimulatory of the extracts acts synergistically with TPA to yield nearly 5 PD per week (Eisinger, Marko & Ogata, 1985).

If melanocyte culture systems are to realize their potential usefulness in skin pharmacology,

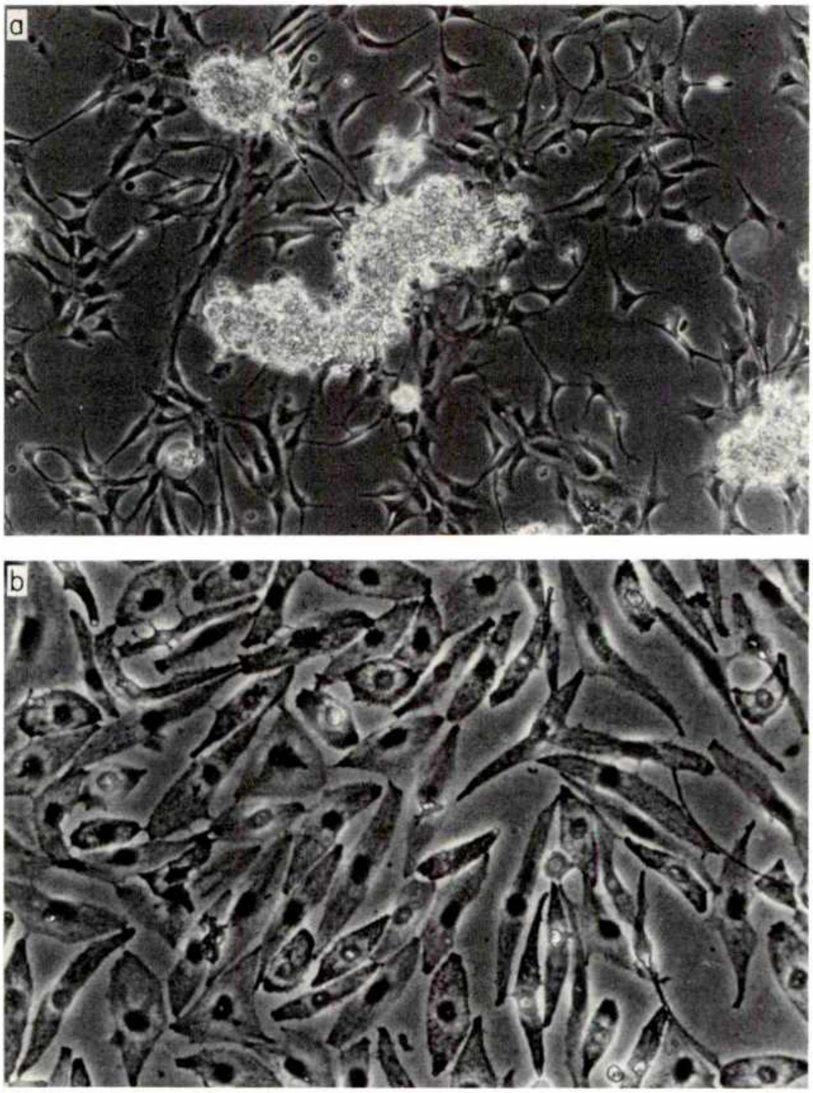

FIGURE 1. Human epidermal melanocytes *in vitro*. (a) After 2 weeks numerous bipolar and tripolar cells are seen surrounding the refractile remains of earlier keratinocyte colonies. (b) First passage newborn melanocytes with polygonal morphology and abundant melanin concentrated at the dendrite tips. No contaminating keratinocyte or fibroblasts are present. (Phase microscopic fields, (a) 400 × and (b) 815 × original magnification).

the cells must manifest not only mitotic responsiveness, but also the ability to synthesize melanin in response to physiological and pharmacological stimuli. To date, substantial baseline melanogenesis *in vitro* has been inferred in all reported systems from serial phase and/or electron microscopic evaluation of the cultures. Early work in our laboratory suggested that ultraviolet irradiation stimulated $^3$H-dopa incorporation by melanocytes in a dose-dependent manner (Wilkins *et al.*, 1982). Eisinger & Marko (1982) reported that cholera toxin increased and TPA decreased the number of melanosomes, as judged qualitatively by electron microscopy. Nielsen & Don (1984), on the basis of 'unpublished data', have reported that melanocyte-stimulating hormone (MSH) also increases pigmentation of cultured human melanocytes by ultrastructural criteria. In apparently contradictory studies, Halaban *et al.* (1983), using the same culture system, found that TPA, dibutyryl cAMP, and isobutylmethyl xanthine increased tyrosinase activity of human melanocytes (found to correlate directly with melanin content of the cultures), while cholera toxin and melanocyte-stimulating hormone had marginal or no effect on the enzyme. Using elegant techniques, these investigators made the further important observations that cultured human melanocytes contain abundant tyrosinase, that the enzyme is synthesized *in vitro*, and that degree of melanization of the cells is directly dependent on the amount (as opposed to the activity) of enzyme present (Halaban *et al.*, 1983). These findings should permit focussing of future *in vitro* studies directed at control of normal and pathological pigmentation.

Finally, with regard to melanogenesis in culture, it should be noted that cells appear to retain their genetically determined *in vivo* behaviour. Specifically, melanosome size and packaging have been noted to depend on donor race in the expected manner (Gilchrest *et al.*, 1984a), and melanocytes cultured from the skin of at least one patient with a distinctive recessively inherited pigmentation disorder were found to contain the same abnormal melanosomes previously described *in vivo* (Griscelli *et al.*, 1978; Gilchrest & Rhodes, unpublished observations).

The major residual problem in cultivation of melanocytes in adequate numbers for pharmacological or other studies is dermal fibroblast contamination. Although not emphasized in publications, it is clear that the dermal-epidermal separation techniques employed in all the published reports, addition of cholera toxin and/or TPA (Eisinger & Marko, 1982) or genticin (Halaban & Alfano, 1984), and attempts at differential centrifugation and/or attachment of skin-derived cells (Eisinger & Marko, 1982; Eisinger *et al.*, 1984; Halaban & Alfano, 1984; Wilkins *et al.*, 1982; Tsuji & Karasek, 1983; Nielsen & Don, 1984) are of limited usefulness, especially in the presence of serum (Gilchrest *et al.*, 1984a; Halaban & Alfano, 1984; and unpublished results). Similarly, 'panning' or immune rosetting techniques cannot be expected to produce the requisite 100% depletion of this hardy cell type from systems otherwise conducive to its growth. Complete success appears to await the development of a culture medium mitogenic for the melanocyte but completely devoid of those factors necessary for fibroblast survival and/or proliferation.

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## DISCUSSION SUMMARY

The following points were discussed with the participation of F.Marks, H.Schaefer, F.Kemper, M.Prunieras and B.Shroot:

(1) Keratinocyte growth factor is heat stable and has a molecular weight under 1000 Daltons. This factor is not the same as the others described, such as E.G.F.

(2) The analogy between living epidermis and *in vivo* keratinocyte culture is very difficult. In culture systems, much more growth occurs: for example, all cells in the basal layer are labelled after 24 h. In skin, modulators reduce the growth rate, but those are lacking in cultures. Another example of this discrepancy is described in psoriasis in which the growth rate increases *in vivo*, but the growth of psoriatic cell cultures has the same rate as normal cells, with doubling every 24 h.

(3) In the work presented, the difference between newborn and aged cells used to establish the culture is evident despite the enormous interindividual variability.

(4) In these cultures, in a defined medium without vitamin A, a small amount of 67 K keratin polypeptide is observed in the cells shed in the medium after 2–3 days; spontaneously detached cells show a consistent amount of this polypeptide.

(5) Eisinger used a combination of TPA and cholera toxin to grow melanoytes. However, it appears that TPA may render cells susceptible to other agents which normally would not act as mitogens. TPA also renders keratinocytes sensitive to prostaglandins. In the work presented the combination of factors is certainly of great importance. To examine their interaction, it is absolutely necessary to consider separately each one of the factors entering in the combination.

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# Evaluation and relevance of skin sensitivity in animal models

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Allergic reactions of an organism to chemicals are summarized in two categories: (a) the circulating antibody-mediated reactions (first three types of Gell and Coombs classification) occurring a few minutes (anaphylatic shock) or a few hours (Arthus reaction) after contact of the sensitized organism with the antigen; and (b) the cell-mediated reactions (type IV: delayed type hypersensitivity) occurring quite a long time (from 24 to 48 h) after the contact of the sensitized organism with the antigen.

The incidence of contact dermatitis in the human population has led to the development of predictive tests in human or in experimental animals for the induction of delayed type hypersensitivity to chemicals.

## DELAYED TYPE HYPERSENSITIVITY

The delayed type hypersensitivity is mediated by sensitized cells resulting from the interaction between the antigen and sensitized T lymphocytes. The antigen passing through the cutaneous barrier activates the lymphocytes. The stimulated T lymphocytes reach a paracortical zone of a regional lymph node via the afferent lymphatics from the periphery. In this thymus-dependent area the lymphocytes are transformed into immunoblasts and differentiated into small effector and memory lymphocyte cells. In this regional lymph node the maximum proliferation is achieved 4 days after antigen stimulation (Turk, 1967). Then the specifically immunocompetent cells are distributed through the whole organism. When there are sufficient sensitized lymphocytes, skin contact with the same antigen will elicit a local cutaneous inflammatory reaction which reaches its maximal response from 24 h to 48 h after contact of the sensitized organism with the antigen.

## PREDICTIVE TESTS

The purpose of the different animal test models proposed is to increase the sensibility of guinea-pigs to chemicals in order to identify their allergic potential. The results obtained have to be relevant and comparable with human reactions.

The scheme of all these tests is based on the following sensitization processes: (a) induction phase by contact of the organism with the compound, and propagation of the immunocompetent cells in the whole organism, and (b) elicitation phase after a contact of the organism with specific sensitizer.

Three categories of methodology are proposed to increase animals' sensibility to chemicals and to avoid as far as possible the different reactivity of the animals compared with human.

### *Multiplication of the contacts between the organism and chemicals by dermal or intradermal routes*

The first predictive test was established by Draize (1959) in the guinea-pig (10 intradermal injections for the induction and a challenge injection after a 2-week rest period). Other experimenters used several applications of the test compound. If the skin reaction scored 24 h after the challenge injection is significantly more intensive than the reactions scored during the induction period, the test material is suspected to be a contact sensitizer.

### *Enhancement of skin penetration*

The purpose of these methods is to increase the test material penetration through the skin barrier layer and to allow contact of the skin living cells with the antigen in physiological conditions. The means used are: (i) chemicals (sodium lauryl sulphate, Tween 80 etc.); (ii) occlusion of the induction site, and (iii) irritation of the induction site by application of irritants, freezing, stripping, abrasion or chemical depilation of the skin.

### *Enhancement of the immunostimulation*

The purpose of these methods is to magnify the guinea-pig immunological response by using adjuvant injections [mineral oil, cyclophosphamide, carrageenan and Complete Freund's Adjuvant (CFA)]. The adjuvant acts on the antigen dissemination and on the stimulation of auxiliary cells and T lymphocyte cells. Different authors have developed new methods using maximizing factors to obtain high sensitivity of guinea-pigs to chemicals.

The guinea-pig maximization test (GMT) introduced by Magnusson and Kligman (1969) is often described as the most sensitive method. This method uses the different maximizing factors: (a) intradermal injecion of the allergen and complete Freund's adjuvant in the dorsal induction site; (b) occlusive application of the allergen on the irritated dorsal induction site followed by a 2-week rest period, and (c) occlusive challenge patch test.

The GMT allows the identification of weak allergens and the results are comparable with human sensitization to many chemicals.

## PERSONAL EXPERIENCE

The question arises whether false-positive responses may be obtained with the GMT, when introducing the test material and adjuvant through the epidermis barrier. An immunogenic hapten-protein complex may be produced by mixing the test material and adjuvant through binding *in vitro* or *in vivo* low molecular weight hapten to the protein of CFA killed mycobacterium; CFA may also facilitate the binding of the hapten with the epidermic proteins.

### *Mitogenic enhanced animal hypersensitivity test (MEAHT)*

*The test was developed in accordance with the following principles:* (a) avoiding false positive responses suspected in the GMT by suppressing the injection of a potential immunogenic complex through the cutaneous barrier, (b) increasing the contact of the organism with chemicals by skin irritation and occlusive patch, and (c) increasing the reactivity of sensitized guinea-pig by *in vivo* multiplication of sensitized T lymphocytes under the action of a mitogenic compound: the phytohaemagglutinin (PHA).

*Working bases.* (a) Maximum proliferation of immunoblast in the regional lymph node is achieved 4 days after antigenic immunostimulation of the ear of guinea-pigs (Turk, 1967); (b) blast cells proliferation in the regional lymph node is observed during the first 5 days after PHA injection in mice paw (Dukor & Dietrich, 1967); (c) *in vitro* T immunostimulant effect of PHA at a 15–75 μg/ml mitogenic concentration on T lymphocyte cultures is observed when applied 48 h after antigenic stimulation (Rich & Pierce, 1973).

### *Preliminary assays*

Different *in vivo* assays were performed in accordance with the working bases. The results were as follows:

*The PHA action in the Draize and GMT tests:* The enhancement of animal reactivity was obtained in the Draize test after a PHA injection at a concentration of 50 μg and 100 μg/ml. The enhancement of animal reactivity was obtained in the GMT after replacement of CFA by PHA at a concentration of 500 μg/ml and no sensitization was observed with PHA at the concentrations of 100, 50, 10 and 5 μg/ml.

*The time of PHA injection after antigenic stimulation.* Positive results were obtained when PHA was injected 48 h after antigenic stimulation.

*The time of challenge patch test after PHA injection.* Positive results were obtained when the challenge was performed on the fifth day after PHA injection.

### *Development of a new animal test method (MEAHT)*

The methodology was developed according to the best results obtained from the preliminary tests (Table 2). (a) The test was performed with 10 male albino guinea-pigs. The test material was used for the induction at the minimal irritant concentration (MIC) and for the challenge at the maximum non-irritant concentration (MNIC). The vehicle was petrolatum or another suitable vehicle. (b) The immunostimulation was performed with Wellcome purified phytohaemagglutinin HA 16/17 at a concentration of 100 μg/ml in saline. Two injections of 0·05 ml were made at the induction site of the dorsal region. Each animal received 0·56 mitogenic unit of PHA. (c) The scoring of cutaneous reactions and allergenicity levels grading of test materials were performed according to the scale of Magnusson & Kligman (1969) (Table 1).

*Scoring.* 0, no reaction; 1, scattered mild redness; 2, moderated and diffused redness; 3, intense redness and swelling.

TABLE 1. Maximization grading

| Sensitization rate (%) | Grade | Classification |
|---|---|---|
| 0–8 | I | Weak |
| 9–28 | II | Mild |
| 29–64 | III | Moderate |
| 65–80 | IV | Strong |
| 81–100 | V | Extreme |

TABLE 2. Mitogenic enhanced animal hypersensitivity test (MEAHT)

| | Procedures |
|---|---|
| *Induction* | |
| Day 1 | Clipping and shaving of the scapula.<br>Application of sodium lauryl sulphate 10% in petrolatum |
| Day 2 | Induction on the scapula:<br>Occlusive patch (4 × 2 cm) of 2 g allergen at the minimal irritant concentration (MIC) |
| Day 3 | |
| Day 4 | |
| Day 5 | |
| Day 6 | Immunostimulation on the scapula:<br>Intradermal injections (2 × 0·05 ml) in the induction site of a mitogenic PHA concentration (100 μg/ml in saline) |
| *Rest period* | |
| Day 7 | |
| Day 8 | |
| Day 9 | |
| *Challenge* | Challenge on the flanks:<br>Occlusive patch test (2 × 2 cm) with 0·2 g of allergen at the maximal non-irritant concentration (MNIC) |
| Day 10 | |
| Day 11 | |
| *Evaluation of the results* | |
| Day 12 | First scoring (24 hours) |
| Day 13 | Second scoring (48 hours) |

PHA, phytohaemagglutinin; MNIC, maximal non-irritant concentration; MIC, minimal irritant concentration.

## *Results*

*Personal testing*. Six test materials were tested at the same concentration with GMT and MEAHT. Three of these were tested also with the Draize test (Table 3). The results obtained with potassium bichromate and *p*-phenylenediamine were similar to the two test methods. With the other test compound, the results obtained were dependent largely on the test method.

*Comparison of our results with other authors*. We compared our results with those obtained with the same compounds by other experimenters and, when possible, with results obtained in human subjects by Kligman (1966) (Table 4).

TABLE 3. Percentage of animals reacting to the 48 h scoring after the challenge patch test

| Test materials and methods | Draize | GMT | MEAHT |
|---|---|---|---|
| *Potassium bichromate* | | | |
| Induction 1% petrolatum<br>Challenge 0·1% petrolatum | 25% (II) | 87% (V) | 100%(V) |
| *Monobenzyl ether of hydroquinone* | | | |
| Induction 1% petrolatum<br>Challenge 0·1% petrolatum | 0% | 80% (IV) | 30% (III) |
| *Procainamide (Pronestyl)* | | | |
| Induction 100 mg/ml saline<br>Challenge 100 mg/ml saline | 0% | 70% (IV) | 40% (III) |
| *p-phenylenediamine* | | | |
| Induction 1% petrolatum<br>Challenge 0·1% petrolatum | — | 80% (IV) | 60% (III) |
| *Benzocaine* | | | |
| Induction 10% petrolatum<br>Challenge 2 5% petrolatum | — | 15% (II) | 70% (IV) |
| *Thioglycerol* | | | |
| Induction 10% petrolatum<br>Challenge 2·5% petrolatum | — | 45% (III) | 100% (V) |

(I) to (V): allergenicity level according to Magnusson and Kligman (1969).

*Comments*

(a) Generally, the sensitivity responses of the guinea-pigs obtained with the Draize test are lower than those obtained with other tests. (b) When comparing our results with those obtained by Magnusson and Kligman (1969) with the GMT on the same test materials, we observed that the allergenicity level was slightly greater in our results. (c) The results obtained with a same test material differs considerably according to the method and the test material concentrations. (d) The MEAHT results sometimes differ from those obtained with other methods but are often similar to the human ones observed by Kligman (1966). Therefore, it can be concluded that the MEAHT is a test method providing, within 13 days, sufficiently relevant results, and that it can be used routinely for the screening of the allergenic potential of chemicals.

In conclusion, the differences observed in the results are due to the test methods, the test material concentration, the vehicle and other factors, as well as the experimenter's evaluation of skin reactions of the sensitized guinea-pigs.

## EVALUATION OF SKIN SENSITIZING REACTIONS

### *Macroscopic observations*

Sensitivity reaction scoring is based on the subjective observation of the skin inflammatory response by evaluation of erythema and oedema. Skin reactions are compared between the test group and the control group. A test material is considered as a sensitizer if the reactions observed in the animals tested are greater than those observed in the control ones.

**TABLE 4.** Comparison of our results with other authors

| | | | Concentrations | | Results (%) |
|---|---|---|---|---|---|
| Compounds | Authors | Methods | I | C | |
| Potassium bichromate | Magnusson & Kligman (1970) | Draize | 1% | 0·1% | 15 (II) |
| | | GMT | 1–1% | 0·1% | 75 (IV) |
| | | human | 2% | 0·25% | 100 (V) |
| Potassium bichromate | Wikström (1962) | Draize | 0·5% | 0·1% | 20 (II) |
| Potassium bichromate | Lombard (1976) | Draize | 0·1% | 0·1% | 25 (II) |
| | | GMT | 1–1% | 0·1% | 87 (V) |
| | | MEAHT | 1% | 0·1% | 100 (V) |
| Monobenzyl ether of hydroquinone | Buehler (1965) | Draize | 1% | 0·1% | 0 (0) |
| | | Buehler | 5% alcool | 5% | 60 (III) |
| Monobenzyl ether of hydroquinone | Magnusson & Kligman (1970) | Draize | 0·1% | 0·5% | 0 (0) |
| | | GMT | 8% | 25% | 50 (III) |
| | | human | 25% | 5% | 12 (II) |
| Monobenzyl ether of hydroquinone | Lombard (1976) | Draize | 0·5% | 0·5% | 0 (0) |
| | | Draize | 8% | 5% | 0 (0) |
| | | GMT | 1–1% | 0·1% | 80 (IV) |
| | | MEAHT | 1% | 0·1% | 30 (III) |
| Procainamide | Lombard (1976) | Draize | 100 mg/ml | 100 mg/ml | 0 (0) |
| | | GMT | 100 mg/ml | 100 mg/ml | 70 (IV) |
| | | MEAHT | 100 mg/ml | 100 mg/ml | 40 (III) |
| Paraphenylene diamine | Buehler (1965) | Draize | 1% | 0·1% | 0 (0) |
| | | Buehler | 2% | 2% | 100 (V) |
| Paraphenylene diamine | Stevens (1967) | Stevens | 10% DMF | 1% | 100 (V) |
| Paraphenylene diamine | Magnusson & Kligman (1970) | human | 10% | 0·5% | 100 (V) |
| Paraphenylene diamine | Lombard (1976) | GMT | 1–1% | 0·1% | 80 (IV) |
| | | MEAHT | 1% | 0·1% | 60 (III) |
| Benzocaïne | Buehler (1965) | Draize | 1% | 0·1% | 0 (0) |
| | | Buehler | 50% acetone | 50% | 20 (0) |
| Benzocaine | Magnusson & Kligman (1970) | Draize | 0·1% | 0·1% | 0 (0) |
| | | GMT | 2–25% | 5% | 28 (II) |
| | | human | 25% | 10% | 22 (II) |
| Benzocaine | Maguire (1973) | Draize | 1% | 0·1% | 0 (0) |
| | | Maguire | 5% | 5% | 25 (II) |
| Benzocaïne | Lombard (1976) | GMT | 10% | 2·5% | 15 (II) |
| | | MEAHT | 10% | 2.5% | 70 (IV) |
| Thioglycerol | Buehler (1965) | Draize | 1% | 0·1% | 0 (0) |
| | | Buehler | 14% | 14% | 60 (III) |
| Thioglycerol | Magnusson & Kligman (1970) | human | 50% | 5% | 100 (V) |
| Thioglycerol | Voss (1958) | Draize | 2·5% | 2·5% | 0 (0) |
| | | human | 1·23% | 1·23% | 10 (II) |
| Thioglycerol | Lombard (1976) | GMT | 10% | 2·5% | 45 (III) |
| | | MEAHT | 10% | 2·5% | 100 (V) |

I, induction allergen concentration; C, challenge allergen concentration. (I) to (V), Magnusson and Kligman allergenicity levels (1969).

Magnusson & Kligman (1969) proposed a scoring of skin reactions based on the evaluation of the redness associated with swelling. But its appreciation is subjective, being dependent on the observer: even in one laboratory it is difficult to standardize the technician's scoring of the sensitizing reactions.

Several methods have been introduced to increase objectivity of the cutaneous reactions scoring: intravenous injection of dyes (Wells, 1972); thermometry (Gloor *et al.*, 1974); infrared photography (Gloor *et al.*, 1974); measurement of the increased skin thickness due to oedema (on the flank or on the paw of tested animals). These methods are not easy to use in routine dermal predictive tests.

A recent method, the mouse-ear swelling test (MEST), seems easier to perform, but has to prove its relevance to human reactions.

### *Microscopic observations*

Histological examinations of the cutaneous response is often performed to differentiate irritation from sensitivity reactions.

*Irritation* is often described as a ballooning of keratinocytes, exocytosis of polynuclear cells and varying degree of epidermal necrosis, and *contact allergic reactions* are often described as mononuclear and basophillic infiltration, intercellular oedema and thickening of the epidermis, vesiculation and spongiosis in the upper dermis. However, one states that the polynuclear infiltrate is also present in low quantity in sensitivity reactions. Some authors state that it is impossible in many cases to differentiate histologically irritant reactions from sensitivity reactions. One has to be careful with microscopic examination because the use of a sub-irritant concentration of the test material for the challenge patch test can associate the picture of an irritant reaction with allergic weak sensitivity reactions.

Other microscopic investigations could be conducted to assess a cutaneous sensitivity reaction: (i) immunofluorescence is a good method to control the quantity of antigenic structures in the epidermis of sensitized animals. This method can only be used in fundamental research, but not routinely. (ii) 'Dermogramme'® has also been used (Duprat *et al.*, 1976): the impression of a fresh sample of skin of a sensitized animal is made on a glass slide and coloured with May-Grunwald. The number of cells is then counted. Allergic cutaneous reactions give skin impressions with a great number of lymphocytes (small and large) and blastoid cells, with a correlative decrease of the other cells. The efficiency of the technique is dependent on the quality of the 'Dermogramme'. This test can be performed routinely and the results are relevant.

## RELEVANCE OF SKIN SENSITIVITY REACTIONS IN ANIMAL MODELS

Apart from the Draize test, the current predictive animal methods for the evaluation of the allergic potential of test materials are sufficiently relevant to be used routinely. Strong and moderate sensitizers are well detected with all proposed tests. Weak sensitizers are only detected with the methods using enhancement of the contact between the organism and test material or using immunostimulating agents to increase the reactivity of guinea-pigs to test materials (Buehler 1985; Magnusson & Kligman, 1969; Lombard, 1976;). The results obtained with weak sensitizers are dependent on numerous factors (animal strain, age, season, test material concentration, vehicle and experimenters). The macroscopic evaluation of the cutaneous reactions is subjective, and the microscopic investigations do not give a clear-cut decision for weak reactions.

We try to avoid these difficulties by using a maximum non-irritant concentration of the test material (MNIC) at the challenge patch test: the control group do not develop any cutaneous reaction and we assume that the cutaneous reactions observed in the test group are provoked by an allergic reaction to the test material. If the test material provoked cutaneous reactions in the control group and in the test group, only well defined reactions in the test group, more marked than the reactions in the control group, are presumed to be due to an allergic response to the test material. Microscopy is used only to characterize doubtful cutaneous reactions.

## CONCLUSION

Considering the numerous factors influencing the sensitivity of the animal test models, it seems unrealistic to presume that predictive animal test models will be sensitive enough to take into account all human variability. The choice of the methodology is mainly a matter of opinion based on the personal experience of the investigators. Each laboratory has to develop its references using its own test methods on various well known sensitizers. It also has to compare them with the same test methods or others performed in different laboratories with the same sensitizers, and when possible to compare with human response.

Maurer (1983) found an approximate incidence of 100 patients per 100 000 persons for a stong allergen, and 10 patients per 100 000 persons for a weak allergen in the general population. Compounds inducing contact dermatitis incidence of 10 patients per 100 000 persons in the whole population will be eliminated by sensitive animal allergenicity test (with adjuvant). Negative results in the guinea-pig mean only that an incidence rate of contact allergy lower than 1/10 000 people may be expected.

After this short review of the evaluation and relevance of skin sensitivity in animal models, it can be assessed that animal testing is not an absolute filter, considering the great variability and heterogenicity of allergic human skin reactivity. The predictive animal models for sensitization must be regarded as only a screening for new compounds to prevent serious accidents to humans.

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## DISCUSSION SUMMARY

The following points were discussed:

The main point discussed was whether animal tests are predictive to man. In fact, it appears that the word 'predictive' should not be used for these tests, which only indicate the ratio of intrinsic sensitizing potential of compounds. It was suggested that attention should be focused on the development of human sensitization studies in volunteers. However, it seems that the tests presently used in man are no more predictive than the tests presently performed in animals.

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# The quail uropygial gland for assays of systemic antiandrogen side-effects of topical drugs

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The function of the sebaceous gland is androgen-dependent. As increased sebaceous gland function is of causal importance in the aetiology of acne and seborrhoea, the usefulness of antiandrogens in the treatment of these diseases is fairly obvious. Because of the effects of antiandrogens on the other androgen target organs, the systemic treatment of males with acne is not possible. The ideal solution would be a topically active antiandrogen without systemic side-effects. We have thus studied the effects of a new topically active antiandrogen, RU 38882 (Secchi, Lecaque & Bouton, 1984).

## METHODS

For this purpose, the uropygial gland of the male Japanese quail was used as a biological model. This gland, a counterpart of mammal sebaceous glands (Jacob & Ziswiler, 1982), was previously shown to be androgen responsive. Its specific response to androgens is an increase of the cellular concentration of androgen receptors (AR) and of dodecane-2,3-diol (C12-diol) in the secretory products (Abalain *et al.*, 1984; Daniel *et al.*, 1984). In order to evaluate the general and undesirable side-effects of the treatment, we controlled the response of the cloacal gland, another androgen target organ, whose weight is drastically increased by androgens. AR were measured by an exchange technique using either $^3$H-R 1881 or $^3$H-mibolerone (Amet *et al.*, in preparation) and C12-diol was measured by a capillary column gas liquid chromatography and expressed in percentage of total diols in uropygial gland secretory products (Abalain *et al.*, 1983).

## RESULTS

Table 1 shows that a single injection of 100 μg of testosterone in the pectoral muscle of castrated quails resulted in the activation of AR in the uropygial gland of the birds. In fact, the activated/unactivated AR ratio rose from 0·4 to 2. The concomitant administration of RU 38882 injected subcutaneously at the level of the uropygial gland inhibited the activation of AR induced by systemic injection of testosterone. This inhibition was dose-dependent and the maximum inhibiting effect was obtained with a single subcutaneous injection of 2·5 mg RU 38882.

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TABLE 1. Inhibition, by single injection of RU 38882, of AR activation induced by testosterone in the uropygial gland

| | | | AR | | |
|---|---|---|---|---|---|
| | | RU 38882 | pmol/mg DNA | | |
| Experimental groups | Testosterone injection | injection (mg) | U | A | A/U |
| 1 | no | no | 2·68 ± 0·2 | 0·69 ± 0·07 | 0·26 ± 0·03 |
| 2 | yes | no | 1·28 ± 0·14$^a$ | 2·62 ± 0·44$^a$ | 2·05 ± 0·41$^a$ |
| 3 | yes | 0·625 | 1·28 ± 0·34$^a$ | 2·0 ± 0·22$^a$ | 1·56 ± 0·44$^a$ |
| 4 | yes | 1·25 | 1·81 ± 0·33$^a$ | 1·23 ± 0·43$^a$ | 0·68 ± 0·27$^a$ |
| 5 | yes | 2·5 | 2·33 ± 0·12$^b$ | 0·87 ± 0·06$^b$ | 0·37 ± 0·02$^b$ |

$^a P < 0·01$ *vs* Group 1.
$^b P > 0·05$ *vs* Group 1.

Various single doses of RU 38882 were injected under the skin close to the uropygial gland of castrated quails (Groups 3, 4, 5). A single dose of 100 μg testosterone was then injected into their pectoral muscle. The quails were killed 1 h later, and their androgen receptors were measured [inactivated (U) and activated (A)]. The results were compared to those obtained in control castrated quails (Group 1) and in castrated quails injected only with 100 μg testosterone (Group 2). Each group consisted of nine birds (means ± SD).

TABLE 2. Effects of long-term treatments of RU 38882 on the response to testosterone of the uropygial gland of castrated quails

| | | | | | Uropygial gland | | |
|---|---|---|---|---|---|---|---|
| | | | Testosterone blood | Cloacal gland | | AR (pmol/mg DNA) | |
| Experimental groups | Testosterone implant | RU 38882 administration | concentration (ng/ml) | weight (mg) | $C_{12}$ diol (%) | Inactivated | Activated |
| 1 | no | no | 0·16 ± 0·03 | 91 ± 7 | 28·7 ± 2·4 | 1·2 ± 0·3 | 0·4 ± 0·2 |
| 2 | yes | no | 3·07 ± 0·61$^a$ | 725 ± 52$^a$ | 39·8 ± 3·8$^a$ | 2·2 ± 0·1$^a$ | 1·2 ± 0·3$^a$ |
| 3 | yes | injected | 3·3 ± 0·8$^a$ | 829 ± 55$^a$ | 30·8 ± 3·9 | 1·0 ± 0·3 | 0·4 ± 0·1 |
| 4 | yes | topically applied | 3·1 ± 0·6$^a$ | 880 ± 50$^a$ | 32·0 ± 3·1$^b$ | 1·2 ± 0·2 | 0·6 ± 0·3 |

$^a P < 0·01$ *vs* Group 1.
$^b P < 0·05$ *vs* Group 1.

All quails were previously castrated. Three groups were implanted with a propionate filled silastic capsule for 21 days. Two of these groups received concomitantly a daily treatment of 2·5 mg RU 38882. In one group (3) the antiandrogen was injected under the skin close to the uropygial gland, and in the other (4) it was topically applied to the skin covering the gland. The results were compared to those obtained in castrated (1) and in castrated and testosterone implanted (2) controls. Each group consisted of nine birds (means ± SD).

Subcutaneous implantation of a testosterone capsule to castrated quails resulted in the enlargement of the cloacal gland (Table 2). Concomitantly, the uropygial gland function was activated, as shown by the increase of its C12-diol production and by the increase of AR, both activation and cell total concentration. The topical application of RU 38882 at 2·5 mg/day for 21 days to the skin covering the uropygial gland inhibited the effects of testosterone on this organ, but not on the cloacal gland which remained fully stimulated. Moreover, in contrast to progesterone and cyproterone acetate, two other inhibitors of androgen action, RU 38882 did not affect the general degradative metabolism of testosterone as demonstrated by the constant level of testosterone blood concentration in the different experimental groups.

## DISCUSSION

We have shown that RU 38882 presents a strong antiandrogenic effect when applied locally to the skin covering the uropygial gland of quails. As already shown in rats (Secchi *et al.*, 1984), this effect is related strictly to the point of application. RU 38882 presents no undesirable systemic antiandrogen side-effects. This compound thus appears to be a good candidate for the treatment of acne and seborrhoea in men.

## ACKNOWLEDGMENT

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# Percutaneous absorption of topically-applied antiandrogens: an assessment with three independent methods

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Orally administered antiandrogens have been used in the therapy of acne and other androgen-dependent skin diseases, but to date, attempts at local treatment have failed. Insufficient bioavailability of the topically applied antiandrogen has been suggested for the failure.

It is believed that drugs applied to the epidermis reach the sebaceous glands—the target organs for antiandrogens—but little information is available on how to optimize the bioavailability of drugs for these skin appendages.

We have therefore assessed penetration into, and absorption from, the skin of laboratory animals, of topically-applied, 3H-labelled antiandrogens cyproterone acetate (CPA) and propylmesterolon (PM). Ethanol and the non-volatile isopropylmyristate (IPM) as a 5% solution in ethanol were used as solvents.

## RESULTS

The distribution of CPA and PM in different skin layers, 4 h after topical application of the substances either in ethanol or in ethanol/IPM (95:5 v/v) was determined in horizontal cryosections (60 μm) of punch biopsies from hairless rats. The results are shown in Figure 1.

Histological evaluation (not shown) revealed that sections 1 and 2 comprised mainly epidermis, whereas the dermis containing the pilosebaceous units was found in sections 3–7. The cutaneous distribution profiles for both CPA and PM in ethanolic solution were characterized by low concentrations in the dermal part of the skin. Most of the applied compound was found in the epidermis (sections 1 and 2).

Addition of the non-volatile IPM to the solvent had a profound influence on the cutaneous distribution profile of both CPA and PM (Fig. 1), indicating an increased accumulation, especially in the region of the dermis where the pilosebaceous units are located (sections 3–7). To determine the corresponding morphology of the dermal accumulation found in horizontal cryosections, histoautoradiography was used.

Figures 2 and 3 show a high density of silver grains over hair follicles and sebaceous glands 3 h after the topical application of the antiandrogens in ethanolic solution to rabbit ear skin. The grain density is enhanced when IPM is added to the solvent (Figs 4 and 5).

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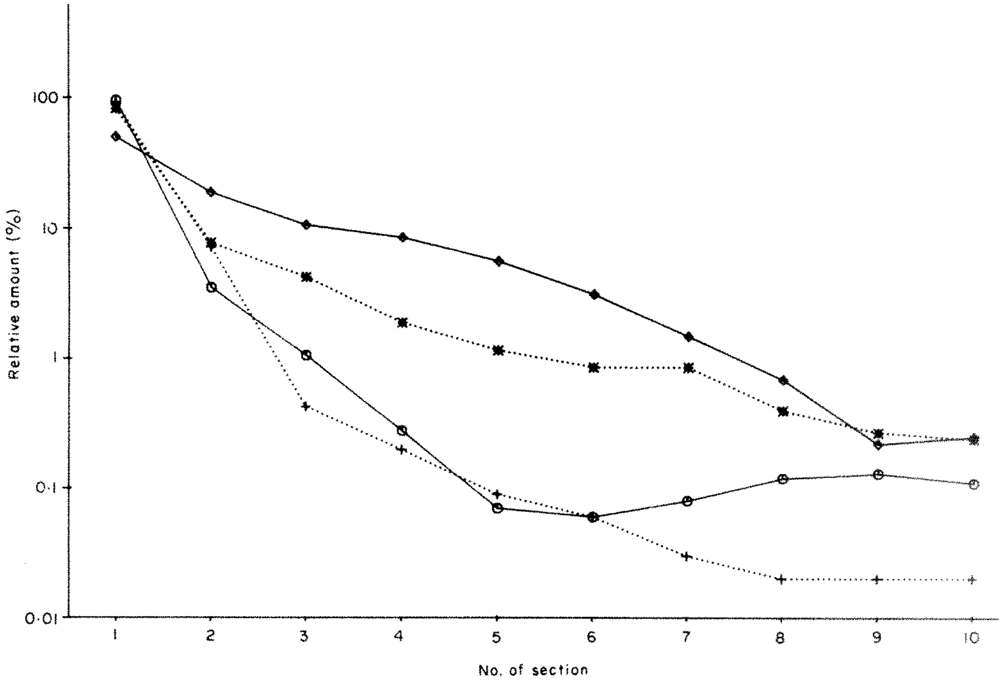
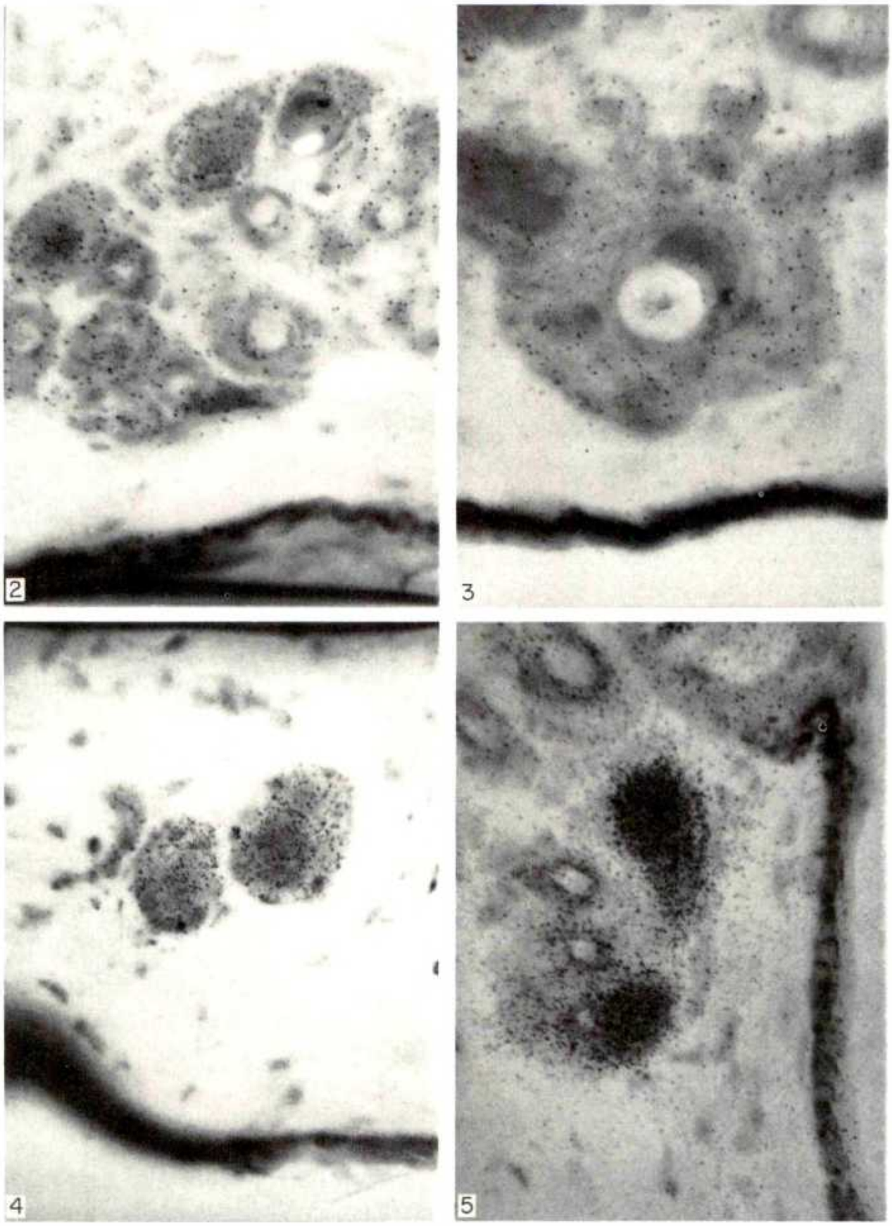


FIGURE 1. Distribution of PM and CPA in cryostat sections of hairless rat skin. Radioactivity was determined in horizontal cryosections of punch biopsies of dorsal skin specimens 4 h after epidermal application of a 1% solution of the antiandrogens. Results expressed as percentage of total amount of substances in 10 sections ($\bar{x}$, $n = 6$). CPA (+) and PM (O) in ethanol. CPA (*) and PM (◊) in ethanol/IPM (95:5, v/v).

This enrichment of CPA and even more pronounced PM in the pilosebaceous units could be confirmed by quantitative evaluation of the histoautoradiographs shown in Figure 6. The difference between PM and CPA could be due to the outstanding lipophilic character of PM (data not shown). Although the concentration of the antiandrogens in the skin increases when IPM is added to the solvent, it could be demonstrated that this effect is not tissue specific. It was therefore of interest to study the percutaneous absorption kinetics into blood vessels which was determined with the isolated, vascular perfused rabbit ear.

A steady increase in the concentration of antiandrogens eventually reaching a plateau was detected in the venous perfusate (Fig. 7). After 3 h, 2.8% (from the applied dose) of PM and 0·9% CPA were accumulated, indicating a higher penetration rate for PM compared with CPA. Addition of 5% IPM to the solvent increased the percutaneous absorption rate of both antiandrogens, but instead of reaching a plateau, the concentration of CPA in the perfusate showed a maximum at 1 h.

In the presence of 5% IPM, 9·6% PM and 7·8% CPA were accumulated from the applied dose into the perfusate 3 h after application. Whether the unexpected absorption profile of CPA in the presence of IPM is due to a transient shunt absorption through the pilosebaceous unit needs further clarification.



FIGURES 2, 3, 4 and 5. Histoautoradiographs from rabbit ear skin 3 h after topical application of the antiandrogens (Sudan III, Hämalaun stain, × 800). CPA (Fig. 2) and PM (Fig. 3) in ethanol. CPA (Fig. 4) and PM (Fig. 5) in ethanol/IPM.

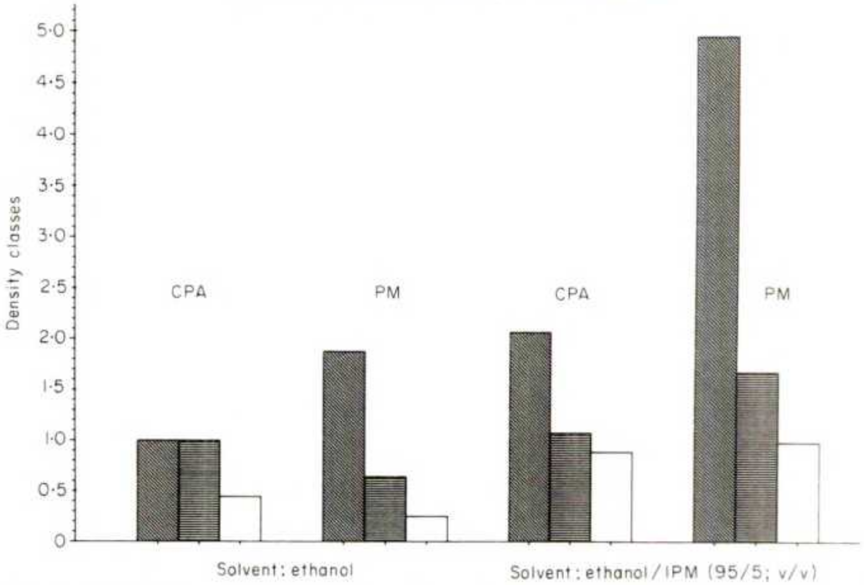


FIGURE 6. Distribution of PM and CPA in rabbit ear skin: quantitative evaluation. Six classes with increasing grain density were defined. The data are evaluated as mean of five areas in 10 pilosebaceous units and the dermis from two rabbits. ▩ Sebaceous glands; ▤ hair follicles; ☐ dermis.

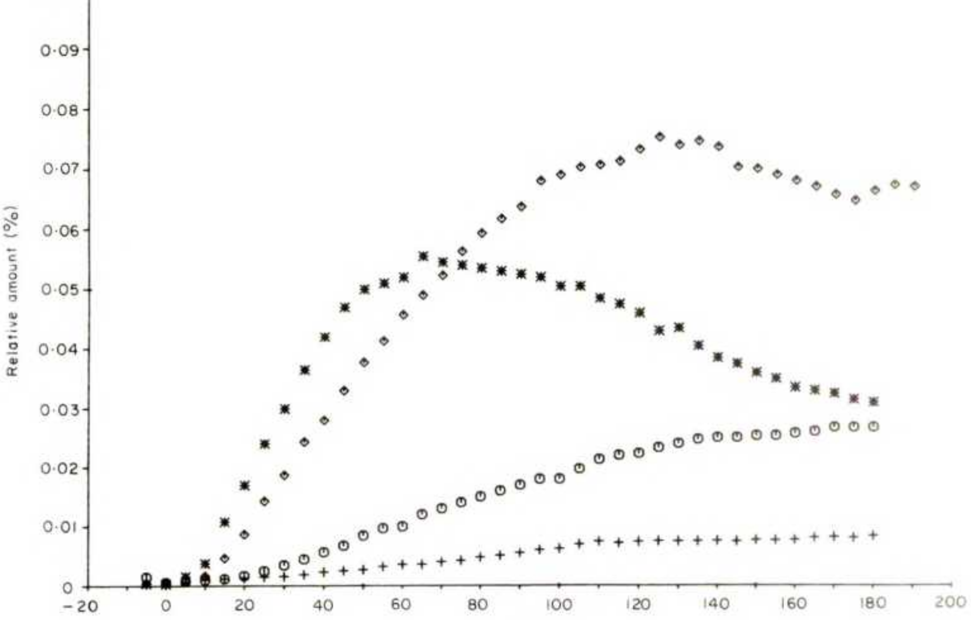


FIGURE 7. Percutaneous absorption kinetics of topically-applied antiandrogens in the isolated, vascular perfused rabbit ear. Radioactivity was determined in the venous perfusate after epidermal application of a 1% solution of the antiandrogens and expressed as percentage in the perfusate of total applied amount. CPA (+) and PM (○) in ethanol. CPA (*) and PM (◊) in ethanol/IPM (95:5, v/v).

## DISCUSSION

The three methods used to evaluate penetration and absorption revealed distinct differences between the topically applied antiandrogens CPA and PM.

Horizontal cryosections and quantitative histoautoradiography revealed an accumulation especially of PM in the pilosebaceous unit of hairless rat skin and rabbit ear skin, which could be enhanced by the addition of the non-volatile IPM to the solvent.

Concomitant to this enhanced accumulation, an increased rate of percutaneous absorption in the vessels of the perfused rabbit ear was observed. The data indicate that an appropriate vehicle can enhance the bio-availability of topically applied antiandrogens in the sebaceous glands. However, these desirable effects are accompanied by an increased absorption into the circulation. It is therefore of special interest that PM in contrast to CPA displays no systemic antiandrogenic effects (Albring *et al.*, 1982; Luderschmidt, Jawny & Eiermann, 1983). An improved clinical efficacy of local antiandrogen therapy can be expected with PM, using an optimized vehicle.

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# Inhibition of wound healing by antiseptics

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Antiseptics and dyes are used widely in the treatment of wounds. Early papers at the beginning of this century described their benefits to wound healing, especially to infected wounds. Only Mobacken and Zederfelt (1973) reported the contrary. They examined the formation of wound granulation in subcutaneously implanted viscose cellulose sponges after treatment with dyes. Their data indicated inhibition, but quantitative histological data were not given. The method described in this paper demonstrates the possibility of a quantitative analysis of wound healing.

## METHODS

Wounds were prepared by incising the dorsal skin of five guinea-pigs (Pibright white strain) in each group down to the muscular fascia. In order to prevent an epithelization, a teflon ring with an inner diameter of 2·0 cm was inserted into the wound and sutured at five points (Fig. 1). The wounds were covered with Geliperm® slab, a polyacrylamide-agar-gel, into which the following aqueous solutions of antiseptics were incorporated: pyoctanin (0·5%), brilliant green (0·5%), eosin (0·5%), chlorhexidine (0·5%), chloramine-T (10%), povidone iodine (5·0%) and $AgNO_3$ (1·0%). Geliperm slab without any active substance was taken for the control group. The wound covering was changed daily. After 7 days, the wound granulation was harvested. Thereafter, histological slices were prepared. As shown in Figure 2, each histological slice was divided into 10 equal parts. Within each part the thickness of the granulation layer, the necrobiotic layer and the fibrin layer was measured. The mean of the 10 values of each layer was calculated and taken as a single value for statistical analysis.

## RESULTS

All substances examined show an inhibition of granulation during wound healing. The strongest effects are to be seen by the dye pyoctanin (Fig. 3), which reduces the granulation layer from 2·32 ± 0·15 mm (control) to 0·12 ± 0·12 mm, corresponding to 5·17% ($P < 0·001$). The data of brilliant green are 5·96% ($P < 0·001$), and those of eosin 83·03% ($P > 0·10$).

Treating the wounds with other antiseptics (Fig. 4), only chlorhexidine produces a strong inhibition. The relative thickness of the granulation layer is 33·77% for chlorhexidine ($P < 0·001$), in comparison to the control (100%), 82·19% ($P > 0·10$) for chloramine-T, 81·38% ($P > 0·10$) for povidone-iodine, and 75·0% ($P > 0·10$) for $AgNO_3$. The thickness of the

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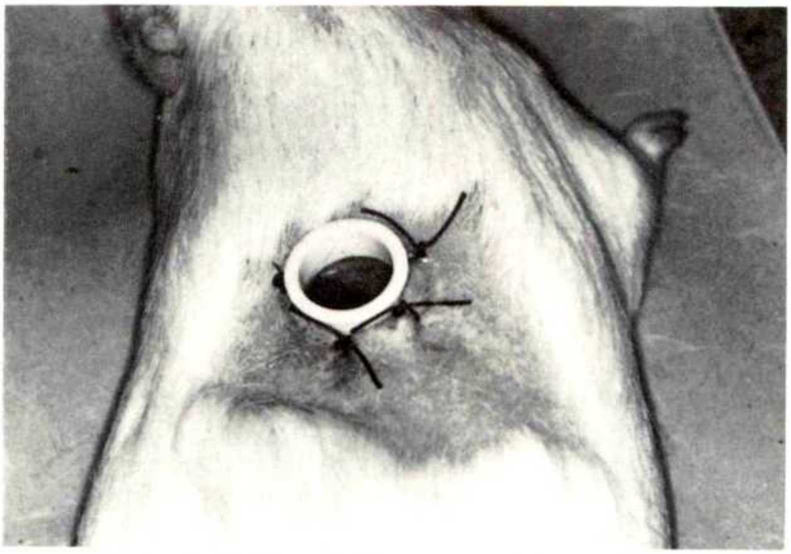

FIGURE 1. Dorsal skin of guinea-pig with teflon ring (inner diameter 2 cm) sutured at five points.

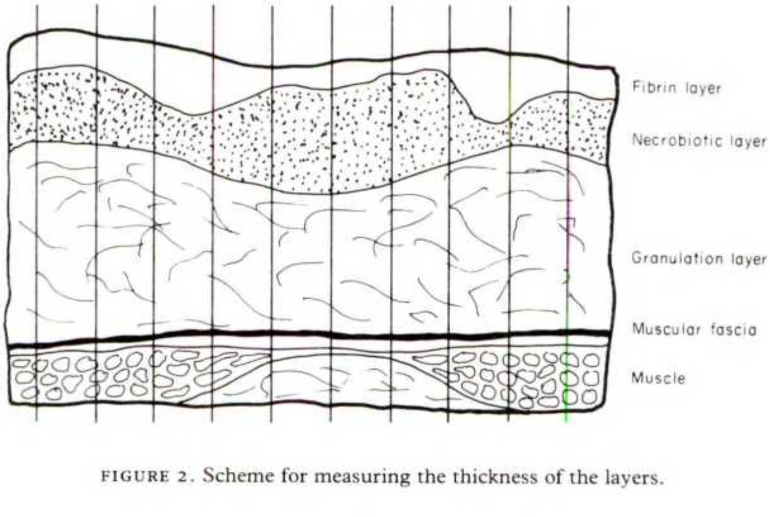

FIGURE 2. Scheme for measuring the thickness of the layers.

necrobiotic layer is reduced in the group of pyoctanin ($P < 0{\cdot}05$) and brilliant green ($P < 0{\cdot}01$). All other substances have no influence on this layer (Fig. 5).

## DISCUSSION

Infected wounds show poor granulation, and therefore to prevent infection antiseptics are used in the treatment of wounds. Not one of the substances examined, applied in concentrations

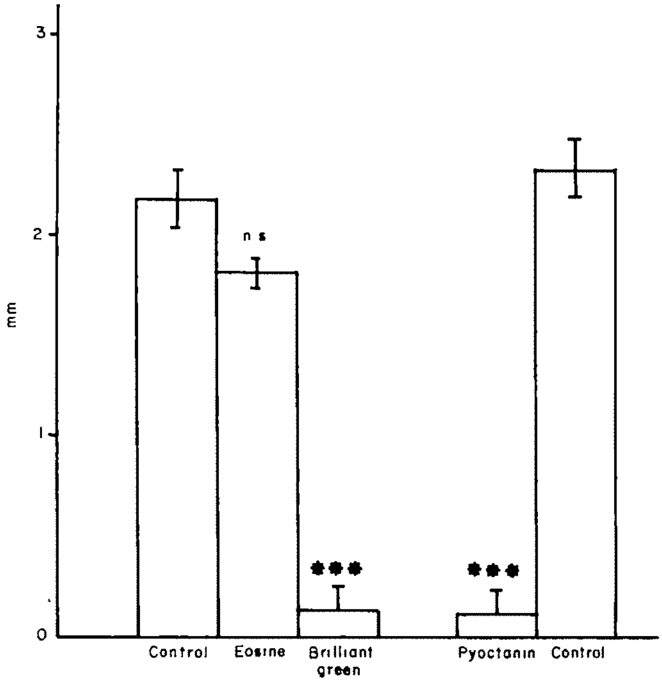

FIGURE 3. Thickness of the granulation layer after the application of dyes. ***, $P < 0{\cdot}001$; n.s., not significant.

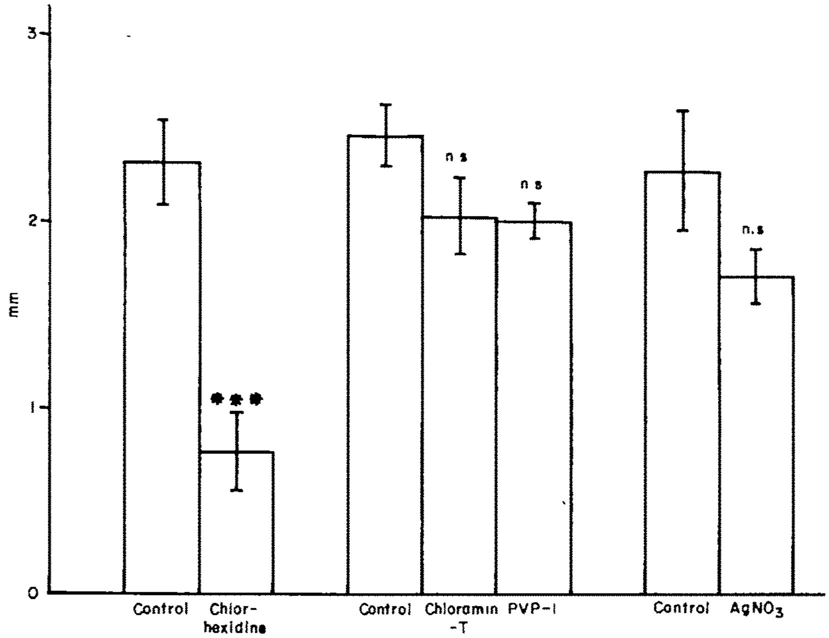

FIGURE 4. Thickness of the granulation layer after the application of antiseptics. ***, $P < 0{\cdot}001$; n.s., not significant.

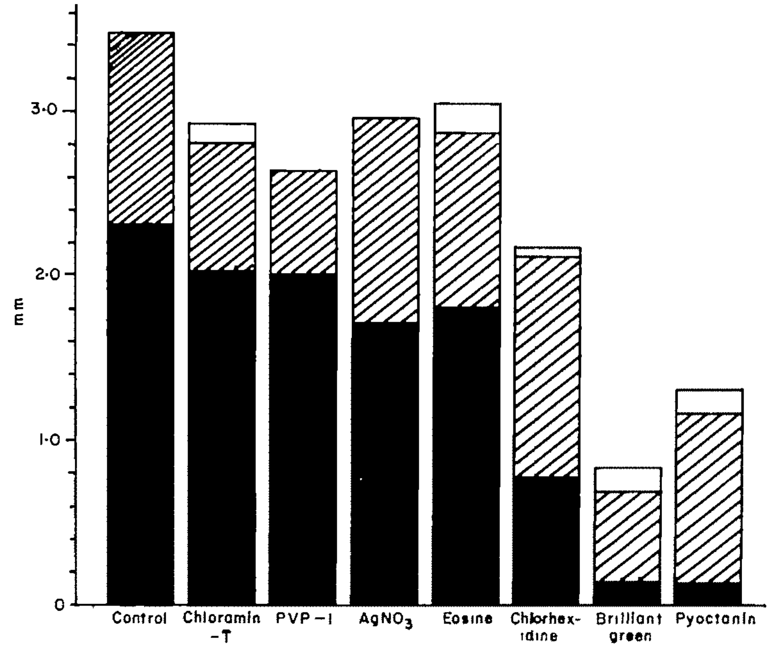

FIGURE 5. Thickness of the different layers. ■ Granulation; ■ necrobiotic; □ fibrin layer.

commonly used in the treatment of wounds, were shown to stimulate the formation of granulation tissue. The slightest, statistically insignificant inhibition is seen by Chloramine-T, PVP-I, eosin, and $AgNO_3$. Chlorhexidine (0·5%) diminishes the thickness of the granulation layer to 33·77%. It is possible that it is not this substance itself that is responsible for the effect, but impurities of the toxic chloraniline. Brilliant green, used as a 0·5% solution, is highly toxic to dividing cells, and the formation of granulation tissue is almost completely inhibited. Pyoctanin 0·5% prevents granulation, a result that corresponds well to *in vitro* experiments with cultures of fibroblasts, carried out by Hagedorn, Kaden & Mittermayer (1979). Triphenylmethane dyes interfere with the DNA of living cells, resulting in a prolongation of the inflammatory phase during wound healing. They inhibit of the formation of collagen and the synthesis of RNA, and they reduce the $O_2$-consumption of granulation tissue (Mobacken, Ahonen & Zederfelt, 1974).

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# The measurement of skin irritation *in vitro* using *ex vivo* rabbit skin

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Skin irritation testing forms an integral part of the overall hazard evaluation of substances and is currently assessed *in vivo* using rabbits (O.E.C.D., 1981). The development and validation of *in vitro* pre-screens to irritation tests has been identified as being important, with the aim of reducing numbers of animals for toxicity experimentation (Balls, Riddell & Worden, 1983), and a number of *in vitro* approaches have been reported which are applicable to the screening of chemicals for potential skin toxicity.

Full thickness (containing a full depth of dermis) skin organ-culture systems have been described (Weissman & Fell, 1962; Raeven & Cox, 1968), but, because of the limitations of nutrient diffusion in a system lacking a blood supply, have used small amounts of tissue. These systems preclude detailed biochemical analysis and, in addition, tissue exposure to test chemical is not limited to normal routes of penetration. Methods of preparing skin slices, consisting of intact epidermis with minimal supportive dermis (approximately 50%), have been developed and used in the assessment of irritant chemicals *in vivo/in vitro* (Middleton, 1981) and *in vitro* (Kao, Hall & Holland, 1983; Oliver & Pemberton, 1985). The morphological structure of 'hairy skin' prevents the preparation of epidermal slices from most laboratory animals, including the rabbit which is the preferred species for *in vivo* studies.

We have developed a technique which uses the full thickness, clipped skin of young rabbits for the assessment of skin irritant potential *in vitro*.

## METHOD

Abdominal skin of young New Zealand rabbits was clipped prior to cleansing with an antibiotic wash solution. Animals were killed by anaesthetic overdose before the excision of areas of full thickness skin with a pinch fold thickness of less than 2 mm.

Skin was aseptically mounted, epidermis uppermost, onto polytetrafluoroethylene tubes and suspended in flow cells containing incubation medium (10 ml), pH 7·4, at 32°C equating with a 5% $CO_2$ in air atmosphere.

Biochemical markers of energy status (tissue ATP concentration), tissue respiration (lactate production) and leakage of selected enzymes into the medium, i.e. aspartate aminotransferase (AST), malate dehydrogenase (MDH) and glutamate dehydrogenase (GLDH), were used to assess the viability of untreated and chemically treated skin during incubation.

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## RESULTS

Control (untreated) skin was maintained *in vitro* under defined conditions for up to 48 h as indicated by less than 20% reduction in tissue ATP levels. During the first 24 h of incubation, the leakage of selected enzymes from control (untreated) tissues was both linear and low (AST, 7%; MDH, 25%; GLDH, 3·5%).

Topical applications of three severe skin irritant chemicals (previously classified in *in vivo* tests) to rabbit skin *in vitro* reduced lactate production by 89% and increased leakage of AST, MDH and GLDH by 2–3-fold above controls in 24 h.

Topical application of three moderate skin irritant chemicals produced less marked inhibition of lactate production (20–55%) and leakage of enzymes when compared to the severe irritants.

Topical application of mild, slight and non-irritant chemicals produced effects which were similar to those seen in control skin.

## DISCUSSION

These preliminary data indicate that the full thickness skin of young rabbits can be maintained *in vitro* in a viable condition for up to 48 h. In addition, the measurement of selected biochemical parameters following chemical application enables moderate to severe skin irritant chemicals to be identified through their cytotoxic action.

Further work is proposed to extend the range and number of skin irritant chemicals tested and the range of biological markers of tissue perturbation to include mediators of the inflammatory process *in vivo*.

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# *In vitro* absorption of 1-chloro 2,4-dinitrobenzene (DNCB) through human, hooded rat and mouse epidermis

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Chemicals which contact skin either accidentally or deliberately may be absorbed percutaneously. Such absorption may elicit a local reaction or a systemic reaction, e.g. salicylic acid (Davies, Vella Britta & Greaves, 1979). Indeed, this has been exploited with the formulation of topical preparations to treat common dermatoses (local responses) and cardiovascular disorders (systemic). 1-chloro 2,4-dinitrobenzene (DNCB) has been used for the topical treatment of papillomas, alopecia and verrucae. Unfortunately, the ensuing systemic exposure, followed by subsequent skin contact with DNCB, can produce hypersensitivity. It has been shown (Carr *et al.*, 1984) that DNCB penetrates human skin *in vivo* and associates with epidermal cells within 1 h of application. Animal models such as the hooded rat and mouse have been used to study the sensitization phenomenon: we have compared the *in vitro* absorption of DNCB through human and the animal model skins.

## METHODS

Epidermal membranes were prepared by a heat separation technique from human skin and by soaking in NaBr solutions for the hooded rat and mouse skins (Scott, Walker & Dugard, 1986). The membranes were mounted in glass diffusion cells (Dugard *et al.*, 1984), and their integrity assessed by measuring tritiated water permeability. The absorption of DNCB was measured through intact membranes. DNCB was applied to the skins in either dimethylformamide (DMF) or acetone:olive oil (A:oo; 4:1 ratio). Samples were taken and assayed by gas chromatography to assess DNCB absorption. Rates of absorption ($\mu g\ cm^{-2}\ h^{-1}$) were calculated and are presented in Table 1.

## RESULTS AND DISCUSSION

The results show that human skin is less permeable to DNCB than hooded rat and mouse skin when applied in the same vehicle (DMF), at the same concentration (10%) and under identical experimental conditions. The rat skin was four times and the mouse skin six times more permeable than the human. When the DNCB was applied to human skin in A:oo vehicle the rate of absorption was much lower than from DMF. This might be due to differences in solubility of

TABLE 1. Absorption* of DNCB through human, hooded rat and mouse epidermis

| | Absorption rate $\mu g\ cm^{-2}\ h^{-1}$* | | | |
|---|---|---|---|---|
| | Human | | Hooded rat | Mouse |
| Vehicle concentration | DMF | A.oo | DMF | DMF |
| 10% | 286·1 ± 52·5<br>($n = 6$) | 8·5 ± 4·3<br>($n = 5$) | 1161·1 ± 227·5<br>($n = 7$) | 1736·7 ± 256·6<br>($n = 5$) |
| 1% | 34·4 ± 4·72<br>($n = 6$) | 2·0 ± 0·5<br>($n = 5$) | | |

* Mean absorpion rate; ± SEM; $n$ = number of determinations.

the DNCB in the two vehicles and subsequent differences in thermodynamic activity of the DNCB, or to the 'accelerant' properties of DMF enhancing absorption through the epidermis. Probably a combination of the two is responsible. When the concentration of DNCB in the DMF was altered from 1% to 10% the rate of absorption through human skin increased almost linearly (8 times greater) but this was not measured after a similar increase in concentration in the A:oo vehicle. This non-linear increase in absorption with the A:oo vehicle further reflects a non-linear increase in activity in the vehicle and and a complex vehicle:solute interaction. The precise reasons have not been elucidated and the present results will be supplemented by further studies.

If development of a sensitization to DNCB was due to the amount of the chemical penetrating the skin then these results would indicate that rodents would react more than humans. Steady state rates, as well as being less, were not attained until after approximately 3 h through the human skin, but were detected after 1·5–2 h through the rodent skins. These differences would indicate a higher systemic exposure to be expected in rodents than in humans following dermal contact with DNCB.

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# Effect of repeated skin administration on *in vivo* percutaneous absorption of drugs

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In clinical situations, topical dermatological therapeutic agents are administered on a repetitive basis. Data available on the effect of regular administration for topical drugs are scarce (Wester & Maibach, 1985). Our objective was to determine the *in vivo* percutaneous absorption of repeatedly applied substances.

## METHODS

Three compounds were chosen: butylated hydroxytoluene (BHT) used as an antiviral agent against herpes, malathion organophosphate used for treatment of head lice, and benzoyl peroxide, a well-known antiacne agent. *In vivo* percutaneous absorption has been investigated after single or multiple applications to the guinea-pig retroauricular bald area (Zackheim & Langs, 1962).

Guinea-pigs, individually housed in metabolic cages, were dosed daily for 15 days (malathion, neat product), 21 days (BHT, solution in Labrafil$^{TM}$ 10%), or 25 days (benzoyl peroxide, solution in acetone or aqueous gel 10%). Once a week, the animals received a radioactive dose of the drug labelled with 14-Carbon. On the other days, non-radioactive material was applied. The application area was either washed or not washed every day 1 h prior to dosing. The washing procedure consisted of wiping the site of application with cotton pads soaked first with a liquid soap-water solution and then with water, followed by the liquid soap-water solution again, and then twice more with water to remove the residual soap.

The absorption was calculated from the amount of radioactivity excreted in the urine, and expressed as a percentage of the applied dose and corrected for excretion by other routes and for retention in the body (Anjo, Feldmann & Maibach, 1980). The BHT data were not corrected.

## RESULTS

Daily malathion application (Fig. 1) without a daily wash produced no change in percutaneous absorption, but daily washing induced an increase of the amount absorbed. The washing effect

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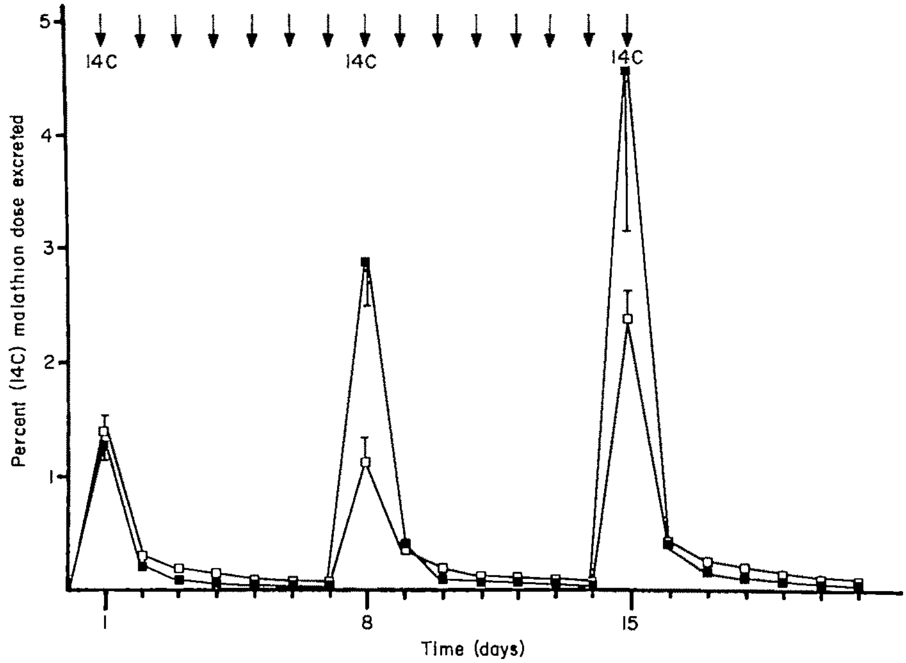

FIGURE 1. Percutaneous absorption of malathion in the guinea-pig following multiple daily applications with (■) and without (□) daily washing. Arrow represents neat malathion dose application; arrow and 14C indicates when radiolabelled malathion was used (adapted from Bucks, Marty & Maibach, 1985).

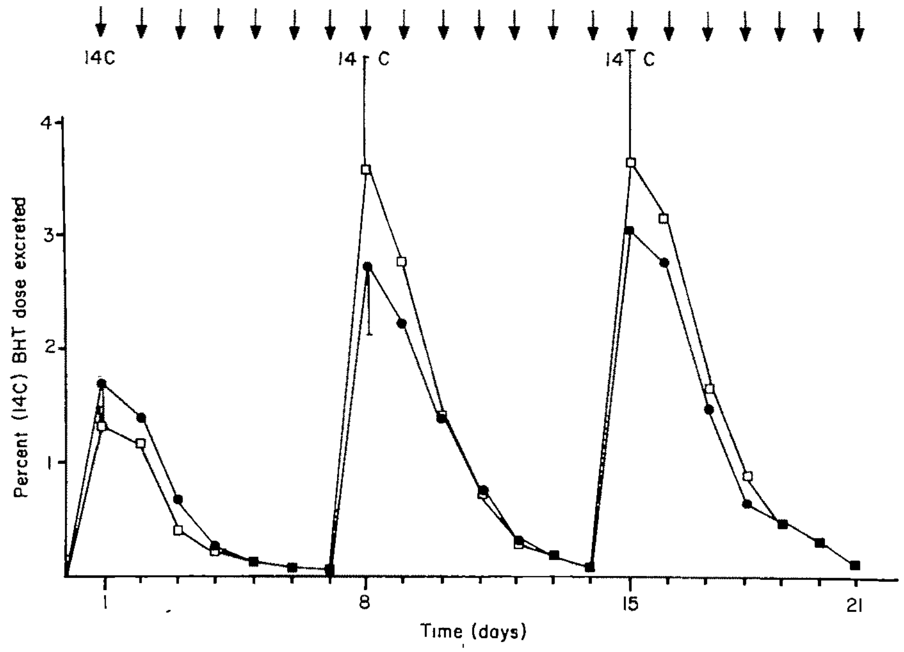

FIGURE 2. Percutaneous absorption of BHT in the guinea-pig following multiple daily applications with (□) and without (●) daily washing. Arrow represents non-radioactive BHT dose application; arrow and 14C indicates when radiolabelled BHT was used (adapted from Courtheoux, Wepierre & Marty, 1985).

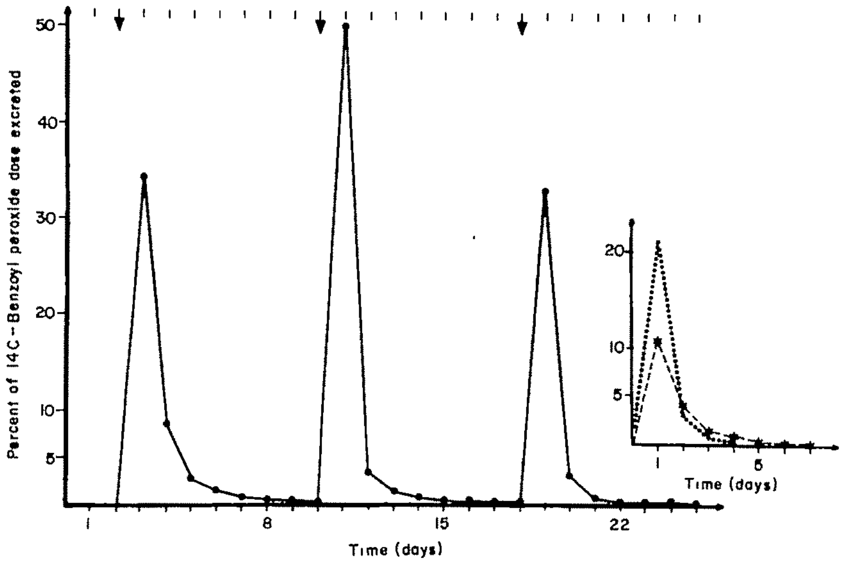

FIGURE 3. Percutaneous absorption of benzoyl peroxide in the guinea-pig. Single application of 14C-benzoyl peroxide in acetone (····) or in the aqueous gel formulation (– – –); the application area is washed 24 h after dosing. Multiple daily dosing and washing, dash represents the application of the gel formulation containing non-radioactive benzoyl peroxide, arrow indicates when the acetone solution of radiolabelled benzoyl peroxide is applied.

with soap and water may be related either to an irritant effect of the detergent (a slight redness of the skin was apparent) or to an increase of the skin hydration.

BHT percutaneous absorption (Fig. 2) increased after long-term administration (8 days), and reached a plateau. This effect could be explained by the following: the slow BHT elimination after administration of a single dose; a pharmacological action of BHT in the skin; and a possible tendency of multiple application of the vehicle to modify lag time and diffusion rate. Removing BHT by washing the skin area every day did not statistically modify the total amount absorbed.

The amount of benzoyl peroxide (dissolved in acetone) absorbed after a primary application of the gel was larger than that obtained after a single application of the acetone solution or of the gel (Fig. 3). After one week of daily treatment with the gel and washing of the skin, the percutaneous absorption increased. The application area showed an irritation which decreased and disappeared after a few days. After 2 weeks of daily treatment, the total amount absorbed from the third radioactive dose decreased. Histological examination of the application area after 21 days showed an acanthotic reaction.

## DISCUSSION

Skin is a viable tissue, able to respond to various topical treatments and drugs. Washing which alters the conditions of the skin can also alter its barrier function. This results in different absorption patterns for subsequent chronic application. Greater percutaneous absorption can occur when a high concentration of chemical is applied to a large body area and remains on the skin for a long period of time. If the treatment modifies the skin barrier properties, absorption can be increased further. Thus, the dosing regimen may have important therapeutical and/or toxicological implications.

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# Models for contact sensitization—novel approaches and future developments

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Contact sensitization (allergic contact dermatitis) due to natural and synthetic chemicals has been recognized for many years. The underlying immunological process of contact sensitization has been elucidated, but many details of the exact mechanisms and controlling features are still to be determined (Polak, 1980; Turk, 1980). In the majority of instances, contact sensitization is regarded as an adverse, and thus a toxic, response (Cronin, 1980). Over the past 50 years, the growth in the science of toxicology and the practice of toxicity testing has witnessed the development of a number of animal models for identifying the sensitization potential of low molecular weight chemicals. The results from these tests contribute to the decisions which have to be made concerning the health and safety of any population which might be exposed to a given material. The purpose of this article is to comment on the current status of animal models and to indicate where future developments in the assessment of sensitization potential may lie.

## CURRENT MODELS FOR CONTACT SENSITIZATION

Some of the more commonly used test methods for assessing contact sensitization are listed in Table 1, and detailed descriptions of their methodology can be found in various reviews (Klecak, 1977; Maurer, 1983). The tests have certain similarties which include the use of the guinea-pig, a biphasic protocol consisting of an induction or sensitiztion phase, followed by an elicitation or challenge phase, the subjective assessment of a skin response as the end-point and an interpretation of sensitization potential based on the incidence of these responses. There are also differences between the tests which include the method of induction and their capacity to identify weak to moderate sensitizers (defined as those agents causing a low incidence response in man either in normal or high risk populations or in human patch test panels). Those methods capable of detecting weak sensitization potential usually require immunostimulation of the animal, e.g. with Freunds Complete Adjuvant.

## MODEL SELECTION

The selection of a test method is based on a number of factors including quantity of available material, urgency for the result, regulatory requirements and, perhaps most importantly, the

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TABLE 1. Models for contact sensitization

| Epicutaneous | Intradermal | Epicutaneous and intradermal |
|---|---|---|
| Stevens | Draize | Split adjuvant* |
| Buehler | Freunds Complete Adjuvant* | Magnusson and Kligman* |
| Open epicutaneous | | Optimization* |

* Freunds Complete Adjuvant used.

required sensitivity (which is related to the size of the exposed population, intimacy and frequency of contact with the product, control over the use of the product etc.). The conservative interpretation of a positive result from a sensitive test model may be equally as damaging as a negative result from a relatively insensitive model, depending on the product type and use. In the first instance, the use of a valuable product may be restricted or rejected. In the second instance, a population may be subjected to an unnecessary hazard or risk.

The Stevens (1967), Buehler (1965) and Magnusson and Kligman (1969) tests are used in our own laboratory for the testing of mainly industrial and agro-chemicals for sensitization potential. The latter two tests are applied when a knowledge of weak or moderate sensitization potential is required and/or when regulatory guidelines are to be satisfied. The Stevens test is the least sensitive, identifying materials of only strong sensitization potential, but it is particularly appropriate for research chemicals, industrial intermediates or products which may be of low volume, highly contained or handled by a trained workforce under controlled conditions. It has the advantage of being a technically simple and rapid assay requiring fewer animals than most of the other tests.

## THE EVOLUTION OF CURRENT METHODS AND THE *STATUS QUO*

Some of the earliest guinea-pig methods for assessing contact sensitization were relatively insensitive and unable to detect moderate or weak sensitizers (Draize, 1959). The need to assess the sensitization potential of products destined for regular, uncontrolled human skin contact where a low response incidence may translate into large numbers of individuals, led to the development of adjuvant tests. Subsequently, the desire for even closer correlation between guinea-pig results and human incidence (Magnusson & Kligman, 1977), together with the need to eliminate the possibility of false-negative results in animals, has resulted in the continued introduction of new protocols (Magnusson & Kligman, 1969; Maurer, Weirich & Hess, 1980), or the modification of more established ones (Oliver & Botham, 1983). Overall, the picture is one of a gain in sensitivity at the expense of increasing the complexity and protractedness of the methodology. Attempts to simplify adjuvant protocols have lead to loss of sensitivity (Goodwin, Crevel & Johnston, 1981). The use of other stimulants of the immune system has also been largely unsuccessful. For example, the intensity of the sensitization response to dinitrofluorobenzene in guinea-pigs is increased and prolonged after pre-treatment with cyclophosphamide (Turk, Polak & Parker, 1976). However, using a Stevens protocol and five sensitizers of different potency, we found that, although pre-treatment with cyclophosphamide increased the sensitivity to dinitrochlorobenzene and sodium chromate, it had no effect on the response to oxazalone, nickel chloride or trichlorophenol (Oliver, Botham & Dugard, 1983).

## FUTURE DEVELOPMENTS AND NOVEL APPROACHES

Current animal tests for contact sensitization have become established within toxicology and have an important predictive role. However, the tests do have limitations and the following improvements could be considered: shortening of experimental protocols and a reduction in their complexity and cost without loss of sensitivity; avoidance of the possible inconsistencies of subjective evaluations; clearer definition between irritation and sensitization responses; economy in the use of animals in testing procedures. However, the empirical design of current animal tests must impose limits on their continued development without a more intimate understanding of the immunological mechanism of contact sensitivity. Therefore, novel approaches which examine the reactive properties of sensitizers and manipulate specific immunological events within the overall process, need to be explored. This view is illustrated below with respect to approaches being taken in our own laboratory and elsewhere, and concentrates on three aspects: hapten-protein conjugation, T-lymphocyte response following antigen recognition, and antigen presentation.

### *Hapten-protein conjugation*

It is generally considered that sensitizing chemicals of low molecular weight must first form high molecular weight adducts within the skin before they can be recognized by the immune system. This principle has been used by Roberts and Williams (1982) who have considered the alkylating ability, lipophilicity and the molar dose administered for specific chemicals in the derivation of a Relative Alkylation Index (RAI). A good correlation between sensitization potential and the RAI was obtained for a series of *p*-nitrobenzoyl compounds and sultones (Roberts & Williams, 1982; Roberts *et al.*, 1983). The authors underline the importance of considering lipophilicity in addition to alkylating ability to obtain agreement with animal data. In addition, a loss in sensitization potential with increasing RAI was predicted, and was interpreted as representing an 'overload' effect *in vivo* which, presumably, could be explained as an immunological tolerance. In most cases, it is probable that, in *in vivo* sensitization tests, this effect would occur at high doses which would be toxic or irritant to skin and would therefore not be achieved in practice if protocols were followed correctly.

### *T lymphocyte response following antigen recognition*

Examination of the predictive value of immune function in the identification of contact allergens has been facilitated considerably by an increased understanding of the nature and regulation of T cell responses in delayed hypersensitivity reactions, and the ability to manipulate such responses by pharmacological intervention. It is now clear that the primary response to hapten is influenced by lymphatic drainage from the site of chemical application and that T lymphocyte activation occurs in the local lymph node(s) (Fray & Wenk, 1957; Turk & Stone, 1963). It is therefore relevant to consider whether T lymphocyte responses in lymph nodes draining the site of chemical application are predictive of the sensitizing potential of that agent.

In the experimental system we describe here, auricular lymph node responses have been examined following the epicutaneous application of chemicals to the ears of BALB/c mice. Animals received either 25 $\mu$l of test chemical in vehicle; dimethylformamide (DMF) or acetone:olive oil (AOO), or an equal volume of vehicle alone. At various periods thereafter lymph nodes were excised, weighed and a single cell suspension of lymph node cells (LNC) prepared under aseptic conditions. The spontaneous proliferative capacity of LNC preparations was determined by incorporation of $^3$H-methyl thymidine during culture for 24 h at 37°C in a humidified atmosphere of 5% CO$_2$ in air.

In preliminary experiments it was observed that topical application of the sensitizer, 2,4-dinitrochlorobenzene (DNCB) caused a time- and dose-dependent induction of LNC proliferation which was associated with an increase in lymph node weight and an increased frequency of pyroninophilic cells. The proliferative response was dependent upon the presence of Lyt-1$^+$ lymphocytes, and histological examination revealed that hyperplasia was restricted to the paracortical region of the lymph node. It is now clear that the antigen-driven proliferation of T cells is dependent upon interleukin-2 (IL-2), a regulatory lymphokine produced following lymphocyte activation (Gillis, Smith & Watson, 1980). We examined whether the addition of an exogenous source of IL-2 would augment the proliferative capacity of hapten-stimulated T cells. Supernatants containing IL-2 were derived from either 12-o-tetradecanoylphorbol-13-acetate (TPA)-treated EL-4 murine thymoma cells (EL-4 IL-2) or from concanavalin-A (Con-A)-stimulated splenocytes (Con-A IL-2). Both sources of IL-2 caused a dose-dependent enhancement of proliferation by LNC isolated from stimulated lymph nodes. As exogenous IL-2 failed to induce the proliferation of control LNC derived from vehicle-treated animals, this lymphokine provides a convenient means of increasing the sensitivity of this model (Table 2).

Employing the method described, we have examined primary T cell responses in the auricular lymph node induced by a variety of nitrobenzene derivatives, including DNCB, 2,4-dinitrofluorobenzene (DNFB), 2,4-dinitrothiocyanobenzene (DNTB) and 2,4-dichloronitrobenzene (DCNB). The sensitizing capacity of all chemicals in BALB/c mice was determined following sensitization with the chemical on the flank (occluded patch) and subsequent challenge to both ears with reduced concentrations. Contact sensitization was measured after 48 h by assessment of (i) ear thickness, (ii) ear weight, (iii) draining (auricular) lymph node weight, and (iv) the proliferative capacity of LNC in the presence and absence of IL-2. The three dinitrobenzene derivatives (DNCB, DNFB and DNTB) were all found to elicit contact sensitization in BALB/c mice. In contrast, at all inducing concentrations examined, DCNB failed to induce a response. Comparison of the primary responses elicited by these chemicals is detailed in Table 3, which clearly demonstrates that, unlike the sensitizing chemicals, DCNB fails to induce an increase in either lymph node weight or LNC proliferation.

A disadvantage of currently available predictive tests for contact sensitizing agents is that the

TABLE 2. Influence of exogenous IL-2 on the proliferative capacity of LNC isolated from DNCB-treated and control (vehicle-treated) BALB/c mice

| Treatment | Medium supplement (10%) | LNC proliferation Mean 3H TdR incorporation ($n = 6$) c.p.m. ± SD × 10$^{-2}$ |
|---|---|---|
| DMF | — | 2·4 ± 0·2 |
| | EL-4 IL-2 | 2·6 ± 0·3 |
| | Con A IL-2 | 2·4 ± 0·4 |
| 5% DNCB | — | 37·3 ± 3·1 |
| | EL-4 IL-2 | 69·4 ± 7·8 |
| | Con A IL-2 | 52·7 ± 4·3 |

TABLE 3. Comparison of the capacity of various nitrobenzene derivatives to elicit primary T-cell activations

| Treatment | Sensitization potential | Mean lymph node weight mg ($n = 6$) | LNC proliferation Mean 3H TdR incorporation ($n = 6$) c.p.m ± SD × $10^{-2}$ | |
|---|---|---|---|---|
| | | | − IL-2 | + IL-2 |
| DMF | − | 2·3 | 4·2 ± 0·5 | 3·9 ± 0·2 |
| 5% DNCB | + | 7·1 | 53·3 ± 3·8 | 92·7 ± 7·2 |
| 5% DNTB | + | 6·8 | 48·7 ± 4·1 | 81·1 ± 3·8 |
| 5% DNFB | + | 8·2 | 81·4 ± 5·2 | 142·2 ± 7·0 |
| 20% DCNB | − | 1·9 | 4·7 ± 0·4 | 5·2 ± 0·6 |
| 10% DCNB | − | 2·0 | 4·3 ± 0·8 | 4·7 ± 0·2 |
| 5% DCNB | − | 1·7 | 3·7 ± 0·3 | 3·6 ± 0·4 |

end-point determination of erythema and/or induration may also be elicited by irritant, non-sensitizing chemicals. We therefore wished to confirm that irritant reactions would not influence the immunological status of the auricular lymph node. Our observations revealed that irritant concentrations of the non-sensitizing chemical sodium dodecyl sulphate failed to cause an increase in either lymph node weight or LNC proliferation.

As indicated earlier in this review, contact sensitivity in experimental systems can be influenced by a number of agents, including cyclophosphamide (Maguire & Ettore, 1967), vitamin A (Miller, Maisey & Malkovsky, 1984) and Freund's Complete Adjuvant (Magnusson & Kligman, 1969). Although in many instances the mechanisms through which potentiated immune function is achieved have yet to be clarified, it is well documented that responsiveness to contact sensitizing chemicals is influenced by a regulatory network of suppressor cells which may exert its effect on both the afferent (induction) phase and efferent (elicitation) phase of contact allergy (Asherson & Zembala, 1975; Turk *et al.*, 1976; Asherson *et al.*, 1977; Dunn *et al.*, 1984). A selective impairment of suppressor cell function in contact sensitivity has been achieved with cyclosphosphamide (Thomas, Watkins & Asherson, 1979; Parker & Turk, 1982), *Corynebacterium parvum* (Knop, Reichmann & Macher, 1981) and interferon (Knop *et al.*, 1984). Of these, the most thoroughly investigated is cyclophosphamide and we have examined the influence of this agent on primary lymph node responses in BALB/c mice. Intraperitoneal administration of 100 mg kg$^{-1}$ cyclophosphamide 2 days prior to sensitization with DNCB was found to cause a significant enhancement of auricular LNC proliferation. Although, as previously described, the capacity of cyclophosphamide to enhance responsiveness may not be of value in all circumstances, the judicious use of selective immunomodulants may provide a means of enhancing primary T lymphocyte responses and facilitate the identification of contact allergens.

Collectively, these data, although preliminary, suggest that the quantitation of murine T-cell activation following topical exposure to chemicals may provide a rapid and relatively inexpensive method for predicting sensitizing potential. It will be necessary to establish the sensitivity of such an approach, but there is already sufficient information to warrant the cautious prediction that methods based upon lymphocyte activation may in the future play a role in allergen identification.

*Antigen presentation in contact sensitivity*

The importance of cutaneous antigen-presenting cells (Langerhans cells) in the induction of contact sensitivity is now widely accepted. The recent elegant experiments of Sullivan, Bergstresser and Streilein (1985) confirmed this belief when they were able to show that pure populations of Langerhans cells, when haptenated *in vitro* with the sensitizing chemical trinitrochlorobenzene and then injected into syngeneic recipients, could induce a contact sensitivity reaction which was of equal intensity to that induced by topical application of the hapten. When Langerhans cells were removed from epidermal cell suspensions and the remaining cells were haptenated, then these cells not only failed to induce contact sensitivity in recipients, but also rendered the recipients specifically unresponsive to a subsequent topical application of the sensitizer.

All antigen-presenting cells, including Langerhans cells, express on their cell surfaces products of the I-region of the major histocompatibility complex (MHC Class II or Ia molecules). Class II molecules have been shown to control the intensity of the immune response to a variety of foreign antigens, including contact sensitizers (Polak, Barnes & Turk, 1968). However, the mechanism of this control process is the subject of considerable controversy. Two hypotheses have been put forward (reviewed by Klein, 1984); the determinant selection hypothesis states that the immune response to an antigen is dependent upon the 'affinity' of the antigen for cell surface class II molecules; the blind spot hypothesis (also known as the clonal deletion hypothesis) states that all antigens can interact with Class II molecules, but that certain T-lymphocyte clones have been either physically deleted or functionally incapacitated.

In the context of determining the contact sensitizing potential of haptens, the determinant selection hypothesis would suggest that the nature of the interaction of a chemical with cutaneous antigen-presenting cells could represent a mechanism by which the immune system discriminates between sensitizers and non-sensitizers.

Contact sensitizing haptens, such as dinitrochlorobenzene, have been shown to bind to epidermal cell proteins. However, it is not clear whether the hapten-protein conjugates thus formed then become associated with Langerhans cells in order to generate the appropriate immunogen for activating T lymphocytes, or whether this can be achieved by direct binding of the hapten to the cell surface. The seminal studies of Landsteiner and Jacobs (1936) showed that for a series of chloro- and nitro-substituted benzenes, only those derivatives which had the capacity to react with aniline (and thus, it was assumed, with protein) were able to sensitize guinea-pigs. However, it remains intriguing why other topically-applied, protein-reactive haptens can sometimes fail to induce an immune response, or even induce immunological tolerance (Iijima & Katz, 1983; Parker, Long & Turk, 1983).

In addition to the need for presentation of haptens in association with MHC Class II molecules on Langerhans cells, there is a requirement for the generation of a second or amplification signal. This signal is an antigen-non-specific factor called Interleukin-1 (IL-1). An IL-1 molecule has been shown to be secreted in the skin by both the Langerhans cell (Sauder, Dinarello & Morhenn, 1984) and the keratinocyte (Luger *et al.*, 1982). Epidermally-derived IL-1 is known as epidermal-cell thymocyte activating factor (ETAF). The stimulus for ETAF secretion by epidermal cells is not known, but could be due to the binding of antigen to critical cell surface receptors.

Work of this laboratory has addressed the role of both the primary interactive signal (the association between hapten and Langerhans cells) and the secondary amplification signal (ETAF) in stimulating a T-cell response in contact sensitivity.

### *A comparison of the interaction of a sensitizer and a non-sensitizer with cells in the skin and draining lymph nodes*

In the BALB/c mouse 2,4-dinitrochlorobenzene (DNCB) is a potent sensitizing chemical whereas the structurally similar 2,4-dichloronitrobenzene (DCNB) is a non-sensitizer when applied topically. Each of these derivatives has the capacity to bind to protein *in vitro*; when incubated in aqueous solution with bovine serum albumin for 6 h at room temperature, the molar substitution ratio was 3:1 for both DNCB and DCNB.

Mice were painted with 500 μg of either DNCB or DCNB and were then sacrificed at regular intervals for up to one week. Skin from the site of application of the compounds (the flank) and the draining lymph nodes were excised, and frozen sections were cut. The sections were stained for the presence of chemical and for Class II-bearing cells by immunofluorescence procedures using monoclonal anti-dinitrophenol and anti-Ia$^d$ antibodies. Within 3h, DNCB was concentrated in the stratum corneum and the epidermis and also around hair follicles; in the epidermis a small number of cells appeared to have on their surfaces particularly high concentrations of the chemical. DNCB-positive cells could be seen in the dermis within 6 h and in the paracortex and medulla of the draining lymph nodes within 24 h. In contrast, DCNB distributed diffusely throughout the skin and DCNB-positive cells were never seen in the dermis or the lymph node at any time from 1 h to one week after application.

The observation of DNCB-positive cells in the dermis coincided with a significant reduction in the number of Class II positive dendritic cells in the epidermis and the apperance in the dermis of somewhat less dendritic Class II-bearing (non-macrophage) cells. Again in contrast, DCNB caused little change in the numbers or distribution of these cells.

It is hypothesized that one pre-requisite of a sensitizing chemical may be an ability to bind to and induce the movement out of the epidermis of Class II-bearing Langerhans cells. The sensitizer then arrives at the draining lymph node on the surface of these cells in a immunogenic form and can stimulate the proliferation of antigen-specific T cells.

Similar observations on the transport of dinitrophenyl groups from the skin to the draining lymph node on the dendritic (veiled) cells have been made by Balfour's group working with the domestic pig (Soeberg *et al.*, 1978; Lens *et al.*, 1983). In addition, they were able to demonstrate that the dinitrophenylated lymph node cells were able to induce contact sensitivity to dinitrofluorobenzene when injected into homozygous recipients.

### *The effect of sensitizing and non-sensitizing chemicals on ETAF production by epidermal cells* in vitro

The possibility that the binding of hapten (or hapten-protein conjugates) to cells in the skin results in the secretion by these cells of the second signal for T-cell proliferation (ETAF) has also been investigated (Botham, 1984).

Epidermal cells were isolated from rat skin by proteolytic digestion and then cultured with a range of concentrations of four different nitrobenzene derivatives: DNCB, DCNB, 2,4-dinitrobenzenesulphonyl chloride (DNBSl) and 1,3-dinitrotoluene (DNT). In the rat DNCB and DNBSl are potent sensitizers, whereas DCNB and DNT are non-sensitizers. The three protein-reactive derivatives (DNCB, DCNB and DNBSl) were also added to cultures as conjugates with rat serum albumin (RSA). Culture supernatants were then dialysed and assayed for ETAF activity using a rat thymocyte proliferation assay.

The results obtained using the optimal concentraion of hapten or hapten-RSA are summarized in Table 4. Significant stimulation of ETAF release was seen after culturing

TABLE 4. The effect of four nitrobenzene derivatives on ETAF release from rat epidermal cells

| | ETAF activity in culture supernatants | |
|---|---|---|
| | *n* | c.p.m. |
| No chemical added | 19 | 812 ± 334 |
| DNCB (10 μM) | 7 | 7208 ± 1395 |
| DNCB-RSA (1 μM*) | 3 | 1153 ± 665 |
| DCNB (10 μM) | 5 | 2836 ± 921 |
| DCNB-RSA (1 μM*) | 3 | 634 ± 471 |
| DNBSl (10 μM) | 5 | 4179 ± 1534 |
| DNBSl-RSA (1 μM*) | 3 | 785 ± 471 |
| DNT | | 898 ± 733 |
| *E. coli* LPS (20 μg/ml) | 12 | 7443 ± 2043 |

* Concentration of hapten in conjugate.
Results are expressed as mean ± SD.

epidermal cells with the three protein-reactive derivatives, including the non-sensitizer DCNB; RSA conjugates did not affect ETAF release at any concentration tested. Subsequent experiments have confirmed that the ability of a chemical to induce the release of ETAF from epidermal cells is not just a characteristic of sensitizers. However, although lacking predictive capacity, the stimulation of ETAF release from epidermal cells may be another pre-requisite for a chemical to cause contact sensitivity in the rodent.

These studies confirm that although the way in which topically-applied chemicals interact with cells in the skin and the draining lymph nodes is critical in determining whether a T lymphocyte response occurs, it is still not possible to advocate a determinant selection hypothesis for the mechanism of contact sensitivity. This will require a more detailed analysis of the interaction between a chemical and the epidermal Langerhans cell, perhaps in particular with cell surface Class II molecules.

## CONCLUSIONS

This presentation has reviewed the current status of animal tests for contact sensitization and illustrated the wide variety of methodology and sensitivity. The need for continued improvement in our ability to identify contact sensitizers has been displayed. Three areas have been discussed, two of which represent novel approaches which merit further examination as methods for assessing sensitization potential, since they offer some advantages over current methods. The third area illustrates the fundamental experimentation which is required within this particular discipline of immunology to provide information necessary for the more rational design of experimental models for identifying the sensitization potential of new chemicals.

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## DISCUSSION SUMMARY

Professor H. Schaefer's remark cautioned Dr Oliver against assuming that the two structurally-related compounds will act similarly biochemically, in that, on the one hand, both DNCB and DNFB will react readily with any kind of amino-acid, thus deactivating any protein receptor on the cell surface, and, on the other hand, DNCB is very quickly biotransformed to highly cytotoxic molecules (dinitrophenol or halogenated acids). Because of this, Professor Schaefer suggested that differences could be explained by simple protein binding of the latter molecules.

Dr Oliver acknowledged these remarks, but added that the sensitizing potential is still observed at least in part, that the two analytical methods used do provide some degree of specificity and, finally, that structurally similar pairs of compounds of this type are difficult to find, especially those having affinity for protein.

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# Short and long-term risks of topical drugs

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Local toxic effects are usually minor and reversible, and are likely to begin with a drying out of the horny layer of the surface of the treated skin. This can be caused directly by the vehicle or its ingredients and indirectly by a pharmacological effect on the sebaceous glands. Usually, the beneficial effects of the vehicle last longer than the negative pharmacological effects, so that no acute irritation of the skin can be seen (Fritz, 1983).

The penetration of both active and auxiliary substances is dependent on the vehicles. Therefore, the contact allergic properties of cetyl alcohol, myristicyl alcohol and lanolin can vary, depending on the composition of the topical product. For contact allergy, penetration is the determinant parameter. In contrast, for skin irritancy the penetration value is unimportant. For this, the concentration in the vehicle and the presence of other possible physically-acting agents are the important factors.

Acute local risks are usually easily detectable during the clinical investigation of new products. Risks can be increased, however, by the method of application (Lange, Nitsche & Zesch, 1981; Nitsche *et al.*, 1984), the condition of the treated skin, and the location of the treated skin, as well as concurrent therapeutic measures (Table 1) and changes in the ingredients of the vehicle. Vehicle ingredients can cause a change in the penetration process of the active substances and thus cause a drying effect on the surface of the horny layer. This can result in chronic damage. Hygroscopic substances [e.g. polyethylene glycol (PEG)], liquid paraffins, lipid solvents (e.g. alcohol and acetone), emulsifiers, propylene glycol and o/w emulsions with large proportions of vaporizable water can cause such chronic damage (Zesch, 1977; 1983).

The drying out process begins with the removal of sebum and oils by emulsifiers, as well as with the removal of natural moistening substances from the horny layer by the ingredients of the vehicle. This drying out can then provoke irritation of the horny layer. Direct irritation of the epidermal cells is usually already detected at the early stages of animal investigations and thus seldom occurs in clinical use.

Summations of local effects can become serious when drying out by an active substance such as benzoyl peroxide is aggravated by the vehicle to such an extent that irritation results. Topically-active substances such as dithranol, Vitamin A acid and 5-flurouracil can cause an acute inflammation at the beginning of treatment. This is often accepted as unavoidable. It is not always clear whether inflammation is accompanied by irritation by the vehicle.

The risk of side-effects caused by active ingredients can be decreased by the reduction of the concentrations used. Although this can, of course, lead to a loss of effectiveness, experience has shown that a markedly longer treatment with a less irritating, less concentrated preparation can often be just as therapeutically effective; for example, topical therapy with a 0·1% dithranol in

TABLE 1. Lindane concentration in the skin (μg/ml tissue) after local application in relation to the penetration period and nature and time of washing of skin with intact stratum corneum

|  |  |  | Concentration (μ/ml tissue) in: |  |  |  |  |
|---|---|---|---|---|---|---|---|
|  |  |  |  |  | Dermis |  |  |
| Washed with | Washed after (h) | Total penetration period | Stratum corneum | Epidermis | Upper | Lower | Subcutaneous fat |
|  |  | 10 | 745 | 0·461 | 0·155 | 0·098 | 0·017 |
| Water/soap | 3 | 10 | 258 | 1·21 | 0·679 | 0·689 | 0·162 |
| Water/soap | 10 | 24 | 178 | 4·67 | 0·893 | 0·67 | 0·0386 |
| Water | 10 | 24 | 378 | 3·27 | 1·52 | 0·657 | 0·345 |

TABLE 2. Different pharmacokinetic behaviours of topical drugs

|  |
|---|
| No first pass-effect |
| Low maximal drug levels |
| Low plasma levels |
| Long elimination periods |

vaseline or a 2–3% benzoyl peroxide preparation can be just as effective as the usual 0·5–1% dithranol ointments or the 5–10% benzoyl peroxide preparations. The lower concentrations result in fewer side-effects.

Serious local damage can also occur directly at, or very near, the treated skin. Usually, such damage is seen only after long periods of time. The topical treatment may already have been discontinued when the damage is observed, so that often no direct connection is made between the damage and the previous treatment. The topical side-effects of corticosteroids were discovered only after 15 years of general use. When such effective active substances are prepared with special penetration-promoting vehicles, the risks become even greater.

At present, there is a trend towards the return to old, proven therapies. Although the use of preparations containing mercury or lead salts is not being practised, there is an intensification of the use of the old tar therapy, and although extensive studies have given no specific proof of a direct relationship between general topical tar therapy and skin cancer in man, we do have proof of such a relationship between treatment of certain areas of the skin with tar and cancer, and between tar therapy in combination with ultraviolet light and cancer. For example, an increased rate of occurence of carcinomas after long-term treatment of scrotal eczema with tar has been found, although other causal factors could not be excluded (Götz, Deichmann & Zabel, 1978).

The penetration of locally-applied substances is much higher in intertriginous areas, as well as some areas of the eyelid and the vulva or scrotum, and thus the risk of cancer in these areas is increased. Even when the treatment of such especially endangered skin areas is avoided, the benefit-risk evaluation for potentially carcinogenic substances should emphasize the safety side (Storer *et al.*, 1984).

Risk of malignant variations of dermal cells after long-term use of Vitamin A acid in connection with ultraviolet light has been suspected. Malignant variations are also suspected to occur after UV light exposure during treatment with tumour-promoting substances such as benzoyl peroxide and dithranol. Animal experiments can be helpful in testing to find out whether such morphological variations can be provoked. However, since the results of experiments on the non-pigmented and hairly animal skin are not generally applicable to human skin, such experiments can only serve to direct our attention to possible risks and their prevention.

Risks have also been connected with photochemotherapy—i.e. UVA therapy in combination with 8-methoxy psoralen. These risks are seldom to the skin itself, since before the introduction of this new therapy special investigations were carried out to determine potential risks from increased UVA radiation to the skin.

Such prophylactic investigations to detect possible long-term risks of topical therapy are extremely difficult since it has not yet been possible to develop a model which can show possible delayed damage to human skin in a short-term experiment. Even the well-known, unfortunately only empirically discovered, skin disorders after corticosteroid therapy are only partly reproducible in clinical experiments. Unforeseen changes are even more difficult to discover at an early stage. These difficulties are even greater when the toxic effects have no relationship to the treated skin, but are rather the result of percutaneous absorption of the active substances. Thus, it is important to consider the kinetics of topically-applied substances since they are quite different from those of the same substance applied by other routes. Low concentration of substances peaking in the serum are followed by prolonged diffusion periods with very low

TABLE 3. Acute and sub-acute salicylic acid intoxication in relation to vehicle, duration of treatment and skin characteristics

| Toxicity of salicyclic acid (acute, sub-acute) | | | | | |
|---|---|---|---|---|---|
| Vehicle | Concentration in the vehicle (%) | Duration of therapy | Concentration in the serum (mg%) | Indication | Clinical symptoms |
| Eucerin | 6 | 11 days | 64 | Psoriasis | Epistaxis<br>Noises in one's ear<br>Deafness |
| Hydrophilic cream | 6 | 4 days (+occlusion) | 47 | Dermatitis | Hallucination |
| Petrolatum | 6 | ? | ? | Erythrodermia (psoriasis) | Generally toxic:<br>tachycardia<br>deafness<br>epistaxis<br>hallucination<br>delirium |
| Eucerin | 0·5 | 20 h | ? | Erythrodermia desquamativa | Fever<br>Hyperventilation (acidose)<br>Dizziness |

TABLE 4. Clinical evidence for chronic salicylic acid intoxication following percutaneous application

| Chronic toxicity of salicylic acid | |
|---|---|
| Suggestive symptoms | Proven symptoms |
| EEG-irregularities | Burning of the oral mucosa |
| Chronic gastritis | Noises in one's ear |
| Dry oral mucosa | Frontal headache |
| | Salicylic acid serum concentration |
| | Salicylic acid urine concentration (e.g. 223 mg%) |

serum concentrations (Table 2). These diffusion periods can last several days since the large take up capacity of the horny layer produces a reservoir effect and the very low transport capability of this layer produces a barrier effect.

Acute damage from active substances is therefore rare. Furthermore, since the acute toxic effects of the active substances are usually known, they are most easily detectable. However, the lack of a first-pass effect of locally applied substances is often overlooked. This is especially true for substances which affect the central nervous system such as salicyclic acid, hexachlorophene and caffeine. Some of these, e.g. salicyclic acid and caffeine, which are relatively non-toxic when applied enterally, are sometimes toxic when applied topically (Luderschmidt & Plewig, 1975;

TABLE 5. Influence of various therapeutic conditions on percutaneous permeation in local therapy involving alterations at the skin surface

| | Percutaneous permeation | | |
|---|---|---|---|
| Surface | Increased permeation | Potentiated permeation | Like-parenteral application |
| Granulation tissue | | | + + |
| Mucosa | | | + + |
| Recent wound surface | | + + | + |
| Dermatitis plus vehicle with accelerants plus occlusion | + | + + | |
| Anogenital area | + | + + | |
| Dermatitis plus accelerants | + | + | |
| Intertrigeneous spaces | + + | + | |
| Dermatitis plus syndets | + + | | |
| Intact skin plus vehicle with accelerants | + | | |

+, Moderate.
+ +, Good.

TABLE 6. Systemic risks resulting from topically-applied drugs, especially when applied on large areas of damaged horny layer

| Active ingredient | Increased by base | Risk |
|---|---|---|
| Salicylic acid | Vaseline<br>w/o emulsion | Neurotoxic<br>Shift in ph |
| Hexachlorophane | | CNS, convulsions |
| Lindane | From fatty-like to o/w emulsion | EEG change |
| Neomycin, framycetin, gentamycin | | Ototoxic<br>Nephrotoxic |
| Tar | Fatty-like | Among others: nephrotoxic |
| Podophyllin plus alcohol (internal) | Tincture | Generally toxic |
| Tannine | | Hepatotoxic |
| Chlorhexidine | | Ototoxic |
| Phenol resorcin | | Among others: nephrotoxic |
| Cds, se-IV-sulphid | | Generally toxic |
| Caffeine | | Convulsions |
| Pyrethrum | | Peripheric nerve<br>Irritation |

TABLE 7. Properties of frequently-employed, topical antiseptic agents with protein denaturing activity

| Protein-denaturalizing substances | Haemostyptic | Antimicrobial | Microbial *in vitro* mutagenicity tests | Scab-forming |
|---|---|---|---|---|
| Heavy metal salts | + + | + | –/+ | + + |
| Tanning agents | + + | — | + | + + |
| Colouring agents | (+) | + | + | + |
| Iodine | (+) | + + | —/+ | (+) |
| Potassium permanganate | (+) | + | —/+ | + |
| Formaldehyde | — | + | + | —/+ |
| Quaternerary ammonium bases | — | + | + | — |
| Syndets | — | + | — | — |

+, Moderately strong.
+ +, Strong.
(+) Mild.
—, None.

TABLE 8. Reasons for non-mutagenicity of pyoctanin under clinical conditions of application, despite certain experimental evidence to the contrary

| Mutagenicity of pyoctanin? | | |
|---|---|---|
| **Realistic situation** | | |
| Topical application on man (1% solution on 1 8 m² skin surface) | Negative | *14 mg/kg/d at 100% percutaneous resorption<br>*0·14 mg/kg/d at 1% percutaneous resorption |
| **Tests for mutagenicity** | | |
| DNA repair tests (bacteria) | Positive | |
| Ames test and other gene-mutagenicity tests (bacteria) | Negative | |
| Drosophila test | Negative | |
| Mammalian cells (*in vitro*) chromosomal mutations (a) below $10^{-6}$ mol/l | Negative | Theoretically: *0·14 mg/kg/d $= 3 \cdot 4 \times 10^{-10}$ mol/l |
| (b) from $10^{-6}$ to $10^{-5}$ mol/l | Positive | |
| Mammals (*in vivo*) chrosomal mutations in bone-marrow cells | Negative | *8 mg/kg/d: oral application |

* Compare these data with the value of 1% percutaneous resorption (0·14 µg/kg/d).

TABLE 9. Factors or experimental results indicating no effect levels

| Assumption of no-effect levels, if: |
|---|
| Natural occurence of the chemical substance or metabolites in the organism |
| No initiating action |
| No genotoxic action |
| No resorption |
| Rapid elimination |
| Metabolism leads to detoxication |
| Non-specific binding to such an extent as to prevent specific binding at the target |
| Pharmacokinetic studies reveal that the substance does not reach the target |
| Carcinogenic effects are dependent on pre-existing tissue damage |

Ippen & Kölmel, 1971). Although it is well known that salicyclic acid has a very high percutaneous absorption rate, this substance is often prescribed in concentrations up to 10% and, in addition, is often used on large areas of the skin. Its wide use is due mainly to its keratin softening properties, which result in better penetration characteristics of concurrently-applied locally active substances. Thus, salicyclic acid itself is viewed wrongly as an auxiliary substance which only affects the horny layer. Case reports in the last few years have shown that patients showing side-effects on the CNS caused by percutaneous salicyclic acid were usually patients with psoriasis. This damage was caused by the often-repeated treatment of large areas of the badly damaged skin (Tables 3 and 4).

Certain broad-spectrum antibiotics are only used topically because of their relatively great nephro- and oto-toxic propeties. Side-effects can also occur, however, when the conditions for percutaneous absorption are favourable, i.e. when the skin is damaged or the horny layer is missing and the effectiveness of the barrier is thereby reduced or lacking (Table 5). For example, when neomycin cream or gauze is applied to burned skin, this can aggravate kidney damage caused by the burn itself. Another example is that when gentamycin cream is applied to skin ulcers, one of the side effects which can occur is deafness (Table 6).

Auxiliary substances such as sodium lauryl sulphate, tween, spans, and propylene glycol can also indirectly increase the risks of potentially toxic substances by increasing the penetration, even on undamaged skin.

Pharmacokinetic characteristics of topically-applied products which often result in very low serum concentration levels make the evaluation of chronic systemic toxicity very difficult. The evaluation of the results of animal experiments on embryotoxicity, teratogenicity, mutagenicity and cancerogenicity is almost impossible. Many substances known to be 'mutagenic' are used externally (Tables 7, 8). In order to allow reasonably reliable conclusions about chronic toxicity, studies must be carried out percutaneously on animal skin. However, such studies are not usually done routinely. The conclusion that a substance is safe because it does not penetrate the skin at all, or only in small amounts, cannot always be accepted, since the duration of the treatment and the effects of concurrent measures are often overlooked.

The low serum concentration peaks, low percutaneous absorption, unknown shoulder levels and the general problems connected with the applicability of animal experiments to man, all prevent one claiming a 'no effect level' with utter certainty (Table 9). Every health professional who prescribes a potentially carcinogenic substance—at any concentration, whatever the penetration characteristics or application methods—should be aware of these dangers, especially since the general public is becoming increasingly sceptical about drug therapies. Fortunately, since adverse effects of topically-applied drugs usually affect the area of the skin treated, they are usually easily detectable. Thus, in spite of the dangers, topical application is usually safer than all other routes of application.

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# Percutaneous absorption of topically-applied drugs in relation to their toxicity

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In terms of toxicity of topically-applied drugs, both cutaneous and systemic effects have to be taken into account.

Major cutaneous effects include irritation, allergenicity, atrophogenicity, comedogenicity, acnegenicity, formation of teleangiectasia and/or pruritus and pain. In terms of systemic toxicity, any side-effects must be taken into consideration, which would occur after slow intravenous infusion. Therefore, the kinetics of percutaneous absorption, distribution metabolism and excretion will govern toxicity.

Penetration studies relative to toxicology after topical application have three aims: to recognize and to quantify the danger, and to indicate how to minimize the risk.

## METHODS AND RESULTS

The horny layer, or in its absence, the epidermis, constitutes the main barrier against percutaneous absorption. Those quantities, which pass through the horny layer and enter into the living layers of the skin and the cutaneous microcirculation, are of relevance for toxicological consideration. The respective methods must therefore reveal this process.

The individual methods or their combination should be predictive and should quantify and identify the drug and follow its kinetics in the skin as well as in the body. Only those methods can be considered as relevant which have been published and confirmed by independent authors, and preferably compared with other methods. Quantification of pharmacological effects such as antimicrobial action, vasoconstriction, vasodilatation etc. are not suitable since potency and quantity enter into the calculation without being separated.

Because of the high barrier effect of the horny layer, the thinness of the skin and, consequently, the low absolute amounts penetrating, most methods are based on radiolabelled drugs, which are expensive. Nevertheless, a hierarchy of methods can be established, which keeps the costs at a reasonable level (Table 1).

In the following, it should be remembered that there is a considerable variabilty in penetration in different skin sites and between different individuals. The same holds, however, for toxic effects also. Thus, one should not expect great precision from these methods, but rather, an order of magnitude which is sufficient for comparison with toxicological data of the same quality.

TABLE 1. Hierarchy of penetration methods

| |
|---|
| Permeation through isolated whole animal skin *in vitro* (orientation) |
| Percutaneous absorption in animals *in vivo* (orientation) |
| Permeation through separated human horny layer (+epidermis) *in vitro* (predictive test) |
| Quantification of horny layer reservoir in man *in vivo* (predictive test) |
| Percutaneous absorption in man *in vivo* (partial balance) |
| Quantification in human skin *in vitro* (complete balance) |

*Permeation through whole animal skin in vitro (Franz, 1975; Bronaugh, 1985)*
Since penetration in human skin cannot be compared directly or indirectly to that in animal skin, this method is only useful for orientation in comparison to chemically-related compounds, or for investigations of the same compound in different vehicles. However, one has to exclude, with certainty that a significant part of the drug in question remains bound to dermal protein (the dermis represents a compartment of 10 times the size of the epidermis), thereby escaping quantification so that the variations of this binding lead to erroneous conclusions.

*Percutaneous absorption in animals in vivo (Bartek, La Budde & Maibach, 1972; Wester & Maibach, 1985)*
This method is specifically useful for studying the distribution, metabolism and excretion of drugs after the kinetics of cutaneous penetration have been established by one of the methods mentioned below. In addition, it permits investigations on effects occuring prior to penetration, i.e. the liberation from the vehicle, eventual degradation on the skin and reactions with horny layer constituents and reservoir. As to the penetration itself, the method provides only an orientation.

*Permeation through separated human horny layer (and epidermis) (Polano et al., 1972)*
As mentioned above, these layers constitute the main barrier. Differences between *in vitro* and *in vivo* diffusion are of minor importance relative to the goal of determining the order of magnitude of the process. Thus, isolated human epidermis is perfectly suited for penetration studies. One might object that a fraction of the permeating substance binding to epidermal protein escapes quantification; however, this will be eliminated towards the skin surface *via* epidermal proliferation and has no bearing on systemic toxicity.

*Quantification of human horny layer reservoir (Rougier et al., 1983; Rougier et al., 1985)*
It could be shown by ourselves that in animals there is a direct and linear correlation between the quantities present in the horny layer 30 min after topical application and the subsequent percutaneous absorption and excretion measured in the same animals over 4 days. These findings have been confirmed by independent investigators. Investigations showing the same relationship in humans have been completed and will be published shortly. The advantage of this procedure lies in the fact that sufficient amounts for non-radiolabelled analytical methods such as HPLC, GC and others can be isolated by a rather inoffensive and painless method in human volunteers, i.e. by a few tape strippings. Provided that our findings are confirmed, this

could become the method of choice. (One should not expect the same relationship to hold between reservoir and percutaneous absorption when the horny layer is pathologically altered.)

### *Percutaneous absorption in man in vivo (Feldman & Maibach, 1969; Wester & Maibach, 1985)*

This is a classic and reliable method. Its restrictions (the calibration *via* bolus injection of the compound in question is sometimes not possible and it does not mimic precisely the very slow, low level penetration in cutaneous processes) are of minor importance relative to the goal of range finding. The major obstacle, i.e. that for the sake of analytical sensitivity the drug has to be applied in a radiolabelled form, is being overcome gradually by the development of highly sensitive 'cold' analytical procedures.

### *Quantification in human skin in vitro (Schaefer, Zesch & Stüttgen, 1982; Schalla & Schaefer, 1985)*

Penetration into a specimen of human skin in a penetration chamber, removal of the horny layer by stripping, subsequent slicing parallel to the surface and quantification in the different skin layers, and an acceptor fluid under the skin allows a complete balance, i.e. kinetic and distribution studies of topically-applied radiolabelled compounds. Because of the thickness of the dermis (0·5–2 mm) relative to that of the epidermis (50–150 μm) and particularly to that of

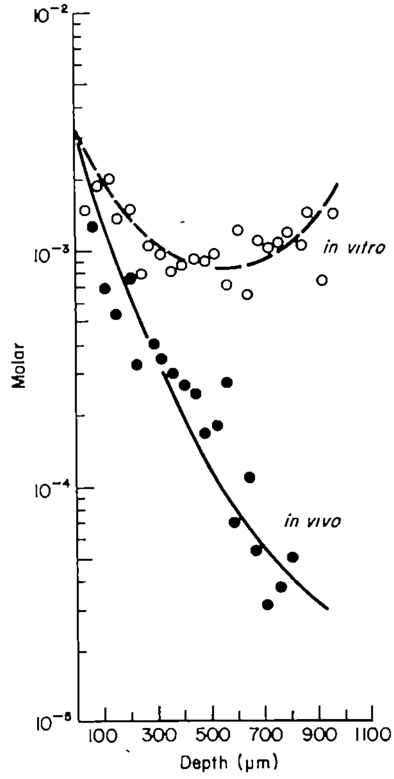

FIGURE 1. Molar distribution of hydrocortisone in human skin ○---○ *in vitro*; ●——● *in vivo* (1% hydrocortisone in polyethyleneglycol ointment; 1000 min penetration period).

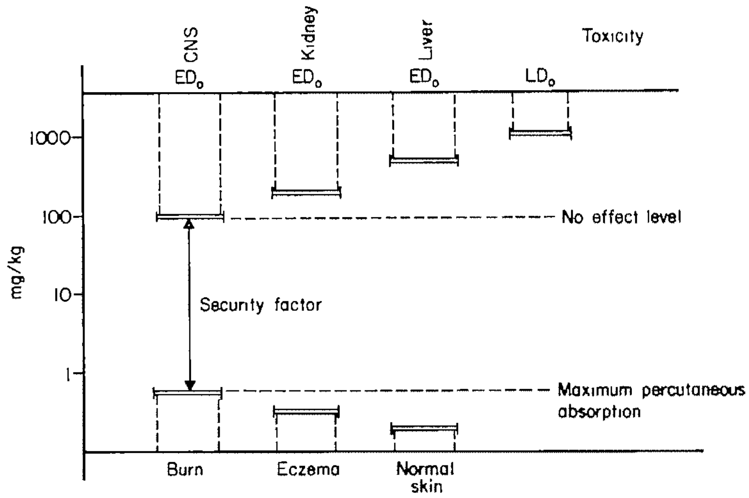

FIGURE 2. Hypothetical illustration of security factor as assessed by comparing systemic dosages causing no toxic effects and maximum cutaneous absorption ($ED_0$ and $LD_0$ are no-effect levels).

the stratum corneum (10–30 μm), the dermal compartment serves under *in vitro* conditions as acceptor since the microcirculation is interrupted. From about 1 h penetration time and more, the accumulation concentrations in the dermis are unrealistically high. For toxicological considerations, however, this distortion relative to the *in vivo* situation is of no importance, since the balance of the drug that has passed through the horny layer into the living layers of the skin is the significant parameter. In fact, the difference between the *in vitro* and the *in vivo* distribution in the skin at a given time indicates the amount which has been absorbed *in vivo* by the vascular drainage (Fig. 1). Thus, measurements of the amounts in the epidermis plus the dermis constitute a simple and reliable method for range finding and risk assessment calculations.

To avoid systemic and cutaneous toxic effects in topical therapy, the drug should be confined to the diseased area as far as possible. Whenever feasible, the surplus should be removed after a relatively short contact time (1 h or less) (Schaefer, 1985).

Penetration data which are not correlated to pharmacological or toxicological data are only of academic interest. Compared with the no-effect level, i.e. the dosage at which animals show no toxic symptoms after systemic application, the quantification of percutaneous absorption allows one to establish a security factor and thereby the risk assessment of topically-applied drugs (Fig. 2).

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## DISCUSSION SUMMARY

The following points were made:

(1) Often the precise quantity of a topical drug actually applied and the levels of this drug ultimately appearing systemically in the organism are unknown, and should be more closely controlled by clinical pharmacologists and dermatologists. However, it should not be necessary to establish more precise data for these drugs applied topically, where in fact the actual tissue levels remain generally unknown.

(2) The safety factor calculations assume that the drug will be applied to the whole surface of the body at one time, under conditions of maximal absorption.

(3) Absorption of a given drug into the organism may not be at its highest in burned skin, since in this case the blood flow in the upper dermis is reduced by stasis. Stripped skin may be a better 'model' for the highest level of percutaneous absorption.

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# Treatment of common and plantar viral warts with human lymphoblastoid interferon-α—pilot studies with intralesional, intramuscular and dermojet injections

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## SUMMARY

Pilot studies with human lymphoblastoid interferon-α (Wellferon$^{TM}$), utilizing the intralesional, intramuscular and dermojet routes, have been performed in 39 patients with common and plantar viral warts resistant to conventional therapy. Clinical efficacy, local and systemic toxicity, and effects on haematological and clinical chemistry parameters has been measured. Our results indicate that the intralesional route is the most favourable as it combines an acceptable therapeutic ratio with a procedure that may be performed quickly and with no prior preparation of the lesion.

Interferons have anti-viral, anti-proliferative and immunomodulatory properties, and thus appear to be a rational choice as a therapy for warts failing to respond to conventional treatment. However, it is clear that interferons have the potential to produce a wide range of side-effects and adverse reactions following systemic absorption, and as viral warts represent a benign, if at times an extremely troublesome disease process, it is of importance to achieve the most favourable therapeutic ratio for this treatment modality.

Following an encouraging pilot study which utilized intralesional injections of human lymphoblastoid interferon-α in seven patients with common and plantar warts resistant to conventional therapy (Gibson & Harvey, 1984), we decided to embark on a series of open pilot studies to evaluate more fully this treatment modality. The aim of these studies was to identify a safe, effective and patient-acceptable treatment regime, which could be used subsequently in fully controlled studies.

In this brief report we concentrate on the toxicological aspects of the studies. Full details relating to this work will be published elsewhere.

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## MATERIALS AND METHODS

Patients aged 16 years or more attending the London Hospital Skin Department with common and/or plantar viral warts of at least one year's duration and resistant to rigorous application of conventional treatment were considered for entry. Patients had not received anti-wart treatment for at least 2 weeks prior to entry into the trial. Human lymphoblastoid interferon-α (Wellferon, Wellcome Foundation Ltd, Beckenham, Kent, U.K.) was used in all of the studies.

In the intralesional study, the largest or most troublesome wart was injected once a week for 12 weeks using a 25 gauge needle and doses ranging between 1·1 and 5·8 mega units (MU) per injection.

Patients in the intramuscular study were divided into 'low dose' and 'high dose' groups, and injections were given into the hip region. Patients in the 'low dose' group were scheduled to receive 5·6 MU per week in two divided doses for a period of 6 weeks. Patients in the 'high dose' group were scheduled to receive 14 MU per week in five divided doses for 2 weeks, and subsequently 10·64 MU per week in two divided doses for an additional period of 2 or more weeks.

In the dermojet study, patients received injections by the dermojet technique (Pan Jet, Schuco International London Ltd, London, U.K.), into or immediately adjacent to the lesions once or twice a week for 6–12 weeks. Common warts were injected without prior preparation, but plantar warts were pared before injection in order to facilitate penetration of the interferon into the warty tissue. In addition, Saran$^{TM}$ wrap was applied as an occlusive dressing to plantar warts for 12–24 h after treatment. The total dose administered depended on the site, size and number of warts present.

In all studies, patients were clinically assessed at least once a week during the treatment period for evidence of clearance of warts and for signs of local and systemic toxicity. Blood for haematological and clinical chemistry analysis was taken at regular intervals throughout the treatment period. After completion of therapy, follow-up assessments at 2 and 6 weeks were performed.

## RESULTS

Sixteen patients entered the intralesional study and, of these, 12 patients (7 males, 5 females) completed an adequate treatment course. Their ages ranged from 19 to 54 years (mean 35 years) and their warts had been present for 1–20 years (mean 4·9 years). The number of injections administered per patient ranged between 5 and 35 (mean 13·7). The total amount of interferon administered per treatment course ranged between 29·3 and 170·1 MU.

Eleven out of the 12 patients achieved complete clearance of their injected warts during the assessment period. Although painful, the injections were usually within the limits of the patients' tolerance. When it occurred, pain associated with injections was short lived and probably associated with a fluid pressure effect. On only one occasion, out of 164 injections, did a local tissue reaction occur. This took the form of swelling and redness in the area of the injection lasting for 5 days. Otherwise, the injection sites were remarkably free of clinically evident tissue reactions and, in particular, tissue necrosis did not occur on any occasion. All patients experienced systemic adverse effects during the study period, although these did not necessarily occur with each injection. These effects varied in severity from mild to very severe and at times forced the patient to seek bed-rest until they abated. Adverse effects usually commenced within 4–6 h of treatment and normally cleared by 16 h post-injection. Adverse reactions included headache, tiredness, fever, shivering and sweating, aching and stiffness in muscles and joints, sore throat, dizziness, depression, diarrhoea and vomiting. Blood analysis (9 patients) did not reveal any treatment-related changes in haematological or clinical chemistry parameters.

Seventeen patients entered the intramuscular study and 16 of them completed an adequate treatment course. In the 'low dose' group there were 12 patients (2 males, 10 females) with ages ranging from 16 to 50 years (mean 24·8 years) and their warts had been present for 1–9 years (mean 2·7 years). The number of injections administered per patient was 12, with the total dose of interferon per patient being 33·6 MU. In this group, two female patients achieved complete clearance of their warts and one female patient achieved almost complete clearance. Nine patients were entered into the 'high dose' group and these included some patients who had been treated previously with the 'low dose' regime. There were four males and five females with ages ranging from 18 to 50 years (mean 27·3 years) and their warts had been present for 1 to 5 years (mean 2·6 years). The number of injections administered per patient ranged between 12 and 30 (mean 19·6) and the total dose of interferon administered per treatment course ranged between 33·6 and 131·88 MU (mean 67·9). In this group, one female patient experienced complete clearance of her warts (she had initially been in the 'low dose' group), and one male patient demonstrated significant partial clearance (clearance of 50% or more of the warty tissue). Overall, therefore, taking both groups into consideration, only five patients produced encouraging results.

The intramuscular injections were found to be highly acceptable to the patients as they did not cause any significant degree of discomfort and were not associated with any clinical evidence of local tissue reactions. All patients experienced some degree of systemic adverse effects during the study period, although they did not necessarily occur with each injection. These adverse effects were similar in quality to those described under 'the intralesional study' above, and surprisingly, the severity of the reactions was of the same order as those previously described for 'the intralesional study'. In the 'low dose' group, blood analysis revealed that a drop in white blood cell count occurred in seven out of the 13 patients, and in three of these cases the count fell below normal limits. In the 'high dose' group all patients demonstrated a drop in their white blood cell count and four patients showed values that were below normal limits. No other changes in haematological or clinical chemistry parameters attributable to treatment occurred during the study period.

Six patients entered the dermojet study, all of whom completed a significant period of treatment. There were two males and four females with ages ranging between 20 and 37 years (mean 28·5 years) and their warts had been present for 1·5 to 14 years (mean 6·3 years). The number of treatment sessions administered per patient (each treatment session could involve several dermojet injections), ranged between 5 and 42 (mean 15).

To date, moderately good results have been obtained with dermojet therapy, falling somewhere between those achieved with the intramuscular regime and those achieved with the intralesional regime. Generally speaking, the dermojet injections were well within the limits of the patients' tolerance, but under circumstances where prior paring of the lesions was necessary to facilitate penetration of the interferon into the warty tissue, e.g. plantar warts, the combination of multiple dermojet injections into thoroughly pared skin causes patient discomfort which at times compares unfavourably with a single intralesional injection. A local tissue reaction occurred in one patient and this took the form of swelling, redness and tenderness in the area of the right ring finger which had been treated. This reaction took 1–2 weeks to settle down.

Three of the patients reported mild 'flu-like adverse effects usually occurring on the day after treatment and consisting of sneezing, muscular aches and lethargy. However, these adverse effects were never more than a slight nuisance to the patient and on no occasion did they remotely approach the severity of effects produced by intralesional and intramuscular injections. It is notable that the doses administered by the dermojet technique were often one order of magnitude greater than those administered by intralesional injections using a needle, and therefore the adverse effects were remarkably slight, although it cannot be ignored that they were present on occasion in three of the six patients. No significant changes were detected in haematological or clinical chemistry parameters.

## DISCUSSION

It would appear that human lymphoblastoid interferon-α injected in a range of concentrations varying from 5·6 to 14·1 MU per ml of vehicle is remarkably free of local tissue reactions. Only two minor tissue reactions occurred during 500–600 treatment sessions administered by the intralesional, intramuscular and dermojet routes, including dermojet injections applied to pared skin which was subsequently occluded with Saran wrap. In particular, no necrosis occurred and the warts which healed did so in virtually all cases with no scarring.

There was no clinically significant difference in quality or quantity between the subjective systemic adverse effects suffered by patients in the intralesional and intramuscular groups. However, the data, although preliminary, strongly suggest that the systemic adverse effects profile of interferon given by dermojet is strikingly less than that by the intralesional and intramuscular routes. The intramuscular regimes produced drops in the white blood count in a significant number of patients. These changes appeared to be dose-dependent.

There is no doubt that interferons are agents with considerable toxic potential, but this should not prevent their use in the treatment of selected patients whose lives are being significantly disturbed by the presence of persistent viral warts. Diligent efforts should be made to refine the treatment regime for such patients, and the evidence from our work indicates that suitable modification of the intralesional route is the most practical way forward.

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# Factors influencing anthralin irritancy

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Attempts to make anthralin less irritant have fallen into two categories. These are those modifying the anthralin molecule, and those aimed at making the skin less responsive to the irritant effects of anthralin. Mustakallio (1979), for example, reported that substitution of the methylene group at the 10 position of the anthracene nucleus by two formylethyl groups resulted in a non-irritant anthralin derivative, which was, however, without anti-psoriatic activity. Pre-treatment of the skin with tar (Schultze *et al.*, 1984; Lawrence, Finnen & Shuster, 1984), erythemogenic UVB and oral PUVA (Juhlin, 1981), and topical anti-oxidants (Finnen, Lawrence & Shuster, 1984) have been reported to reduce the irritant effects of a subsequent anthralin challenge. Systemic pre-treatment with prednisolone (Juhlin, 1981), chlorpheniramine and aspirin (Misch *et al.*, 1981), did not affect anthralin irritancy, but indomethacin has been shown to reduce subsequent anthralin-induced inflammation (Kingston & Marks, 1983).

The purpose of the present study was to investigate methods of modifying anthralin irritancy which might be applicable to clinical therapy for psoriasis. The influence of pre-treating the skin with 5% crude coal tar in white petrolatum 24 h before anthralin challenge, and pre-treatment with suberythemogenic UVB (90% of the predetermined minimal erythemal dose) immediately before, and 24 h before, anthralin challenge were tested in a series of bilateral paired comparisons in groups of 10 subjects. Once the irritant response was initiated, 0·5% triamcinolone acetonide in white petrolatum was applied to determine whether anthralin-induced oedema could be reduced. In a further group, anthralin was applied for 1 h and 24 h to determine the effect of reducing the skin contact time.

Twenty-five microlitres of acetone solutions containing anthralin (concentration range 0·01%–1·0%) were evaporated on to 8 mm paper discs which were placed on both forearms under Finn chamber occlusion for 24 h. One forearm acted as a control and the other was treated with the test factor. The increase in skin thickness produced by anthralin 24 and 48 h after application was measured with vernier calipers as a means of quantifying the inflammatory response.

It was found that pre-treatment with coal tar and suberythemogenic UVB did not reduce anthralin irritation significantly, but reducing the anthralin contact time from 24 h to 1 h did (Fig. 1). Once the irritant reaction had been initiated, treatment with triamcinolone acetonide 0·5% reduced skin oedema formation, but not to a degree that reached statistical significance ($P = 0{\cdot}1$, 2-way ANOVA). It is possible that the use of a more potent topical corticosteroid would be more effective. Coal tar, topical corticosteroids and ultraviolet light are often used together in the treatment of psoriasis, and whilst tar and UVB at suberythemogenic doses did

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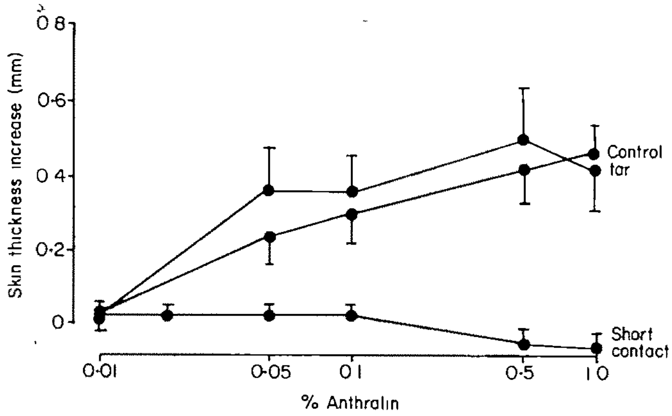

FIGURE 1. Irritant reactions to anthralin in control, tar pre-treated and 1 h contact subjects ($n = 10$; bars indicate standard error).

not lessen the irritant response to anthralin, these treatments did not increase the inflammatory response.

The most effective method found to reduce anthralin irritancy was the reduction of skin contact time. Other studies (Runne & Kunze, 1982) have shown that short contact therapy is successful in treating psoriasis, and thus a 'short contact' regimen should be both effective and less irritant.

## ACKNOWLEDGMENT

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# Treatment of patients with psoriasis by PUVA therapy and mechlorethamine

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PUVA therapy has been used since 1974 to treat patients with psoriasis (Parrish *et al.*, 1974). Topical application of mechlorethamine has proved to be efficient in the treatment of psoriasis. However, although this is a treatment which is easily applied by patients themselves, the major disadvantage of mechlorethamine is related to the high incidence of allergic contact dermatitis. In patients with psoriasis, sensitization of mechlorethamine may be prevented by UVB (Nusbaum *et al.*, 1983) or by PUVA therapy, as we demonstrated in a former study (Mauduit, Silvestre & Thivolet, 1985).

In the present study, we tried to find a convenient schedule for patients, using a combination of PUVA therapy and mechlorethamine.

## METHODS

Two groups of patients with psoriasis (plaque type or guttata) were studied: Group A (20 patients) treated with mechlorethamine alone, and Group B (30 patients) treated by PUVA prior to mechlorethamine.

Both groups were similar with respect to age, and to type and duration of psoriasis. None of the patients had ever been treated with mechlorethamine previously. Patients treated with PUVA therapy, UVB, etretinate or methotrexate during the preceding 3 months were excluded.

In Group A, patients applied mechlorethamine hydrochloride (20 mg%, aqueous solution) on psoriatic lesions daily. In Group B, patients received 10 PUVA treatments, after which mechlorethamine alone was applied daily. Patients were examined weekly for 10 weeks. If clinical evidence of sensitization developed, mechlorethamine applications were discontinued and, 2 weeks later, open and closed patch tests with mechlorethamine 0·02% were applied to uninvolved skin.

## RESULTS

The results of the treatment in Group A are summarized in Table 1. Signs suggestive of contact dermatitis resulted in discontinuation of the treatment with mechlorethamine in 15 out of 20 patients. Fourteen closed patch tests were done (one patient dropped out), 11 of which were positive (Table 2). The open patch tests were all negative. Allergic dermatitis appeared after an average number of 25 applications of mechlorethamine (Table 3).

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TABLE 1. Group A: 20 patients treated with mechlorethamine alone. Efficiency of treatment.

| | No. of patients | % | Mean number of mechlorethamine applications |
|---|---|---|---|
| Improvement 100% | 2 | 10 | 23–34 (28·5) |
| Improvement 80–100% | 4 | 20 | 28–44 (38) |
| Improvement <80% | 9 | 45 | 18–22 (22) |
| No improvement | 4 | 20 | 10–56 (24) |
| Deterioration | 1 | 5 | 42 |

TABLE 2. Contact dermatitis to mechlorethamine in Groups A (mechlorethamine alone) and B (PUVA prior to mechlorethamine)

| | Group A (20 patients) | Group B (30 patients) |
|---|---|---|
| Contact dermatitis | 15 | 7 |
| Allergic dermatitis | 11 | 6* |
| Irritant dermatitis | 3 | 1 |

* $X^2 = 14{\cdot}88$, $P < 0{\cdot}001$.

TABLE 3. Mean number of mechlorethamine applications before sensitization in Group A (mechlorethamine alone) and in Group B (PUVA prior to mechlorethamine)

| | Group A | Group B |
|---|---|---|
| Contact dermatitis | 27 | 31 6 |
| Allergic dermatitis | 25 | 33·7 |
| Irritant dermatitis | 30 | 20 |

The results of the treatment in Group B are summarized in Table 4. Treatment had to be discontinued in seven cases. Closed patch tests were positive in six cases. Open patch tests were all negative. Allergic dermatitis appeared after an average number of 33·7 applications of mechlorethamine.

Comparison of the results in the two groups showed that PUVA therapy decreased significantly the incidence of allergic dermatitis to mechlorethamine ($X^2 = 14{\cdot}88$, $P < 0{\cdot}001$).

TABLE 4. Group B: 30 patients treated with PUVA therapy prior to mechlorethamine. Efficiency of treatment

| | No. of patients | % | No. of PUVA treatments | No. of $J/cm^2$ | Mean number of mechlorethamine applications |
|---|---|---|---|---|---|
| Improvement 100% | 15 | 62·5 | 10 | 36 | 34·6 |
| Improvement 80–100% | 2 | 8·3 | 11·5 | 37·5 | 53 5 |
| Improvement <80% | 3 | 12·5 | 10 3 | 32·3 | 39·3 |
| No improvement | 4 | 16·6 | 10 | 37·8 | 41·5 |
| Deterioration | 0 | 0 | — | — | — |

Moreover, the mean number of mechlorethamine applications inducing sensitization was much higher in Group B compared with Group A.

## DISCUSSION

Our data show that the combined use of PUVA and mechlorethamine may prevent the development of sensitization to mechlorethamine. In patients sensitized to mechlorethamine the time required for sensitization was increased by PUVA.

With respect to the prevention of allergic dermatitis to mechlorethamine, we obtained similar results with PUVA to those obtained by Nusbaum *et al.* (1983) with UVB, and we did not find the combination of PUVA and mechlorethamine more efficient than PUVA alone (Ortonne, Coussa & Thivolet, 1977). It should be emphasized that the UV doses administered before applying mechlorethamine are rather low, as compared to those used in animals to prevent sensitization (Bergstresser, Toews & Streilein, 1980).

The major problem of both PUVA and mechlorethamine is the development of cutaneous malignancies. This had been shown to occur with mechlorethamine in patients with mycosis fungoïdes (du Vivier *et al.*, 1978). Similar lesions have been reported in patients with psoriasis treated with PUVA therapy (Stern *et al.*, 1984). However, these lesions were easily treated. A disadvantage of PUVA therapy is that patients have to attend specialized centres for treatment regularly. PUVA combined with mechlorethamine may be used when PUVA alone is not convenient.

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# Cutaneous phototoxicity reactions

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## SUMMARY

This paper reviews some potential mechanisms of cutaneous phototoxicity and discusses selected methods of predicting and ranking phototoxic reactions.

Phototoxicity is a non-immunological reaction that is induced by the action of light on a photoactive chemical. The phototoxic chemical may be either exogenous or endogenous, and if exogenous may include topical or systemic agents. Examples of phototoxic agents include psoralens (e.g. 5-methoxypsoralen, 8-methoxypsoralen, as well as the isopsoralens). Psoralens are used therapeutically for the treatment of different skin diseases and psoralen-like chemicals may be present in perfumes and cosmetics.

Phototoxic reactions can be evaluated visually in animals. The hairless mouse has also proved useful with skin thickening as a marker of dermal oedema and the induction of polyamine biosynthetic enzymes, as well as other biochemical changes being indicative of cutaneous phototoxicity.

*In vitro* tests of phototoxicity include the *Candida albicans* assay as well as bacterial mutagenicity. For example, in the latter category different psoralens vary in their ability to induce mutagenicity in *Salmonella typhimurium* strains, an assay which can provide rapid screening of potential phototoxicity of chemicals.

For human testing, phototoxic chemicals can be delivered topically or orally, and in conjunction with appropriate ultraviolet radiation will produce phototoxic reactions which can be evaluated for intensity of response.

Human exposure to phototoxic chemicals or drugs may result from either endogenous production of the phototoxic chemical, e.g. in porphyria cutanea tarda, or from systemic and topical drugs such as thiazides, 8-psoralens or coal tars. Exposure to phototoxic chemicals may also occur as a result of cosmetic or perfume application, as seen with bergapten-(5-methoxypsoralen) containing perfumes. Some suntanning preparations available in Europe also contain psoralens as tan-enhancing agents, and use of these may result in undesired phototoxicity reactions. There are several proposed mechanisms of phototoxicity and different phototoxic chemicals may produce phototoxicity by more than one of these mechanisms.

Phototoxic reactions have been divided into type I photo-addition reactions, where the substance upon UV irradiation undergoes direct photo-addition to cell constituents such as proteins or nucleic acids (Song & Tapley, 1979), and type II reactions, which involve reaction with molecular oxygen to form singlet oxygen and superoxide anion. These reaction products can then lead to oxidation of cell membrane components such as lipids and proteins. One example of this is protoporphyrin photosensitization (Lamola, Yamane & Trozzolo, 1973).

Some phototoxic agents will produce both type I and type II reactions. Psoralens can intercalate between adjacent DNA strands and photoreact to form mono-adducts and cross-links between the strands (Blum, 1964; Song & Tapley, 1979). In addition, 8-methoxypsoralen (8-MOP) plus UVA (320–400 nm) has been shown to generate oxygen radicals that can induce lipid peroxidation (de Mol & Beigersbergen van Henegouwen, 1979). The combination of 8-MOP and UVA has also been shown to deplete cutaneous glutathione readily (Wheeler *et al.*, 1985). Glutathione is a non-protein thiol important in protecting cells from oxygen radicals and other reactive chemicals.

The role of inflammatory mediators in phototoxic reactions is not clear at present. While 8-MOP and UVA were shown to enhance prostaglandin biosynthesis in the skin *in vitro* (Lord *et al.*, 1976), they failed to increase skin levels *in vivo* of certain inflammatory eicosanoids (Warin, 1978). Phototoxic reactions should occur in 100% of a population exposed to sufficient amount of the drug or chemical and the appropriate amount and wavelengths of light.

Action spectrum studies of different phototoxic agents have been conducted using a variety of monochromators. The peak action spectra for 8-MOP and TMP appears to be between 335 and 340 nm (Cripps, Lowe & Lerner, 1982), though previous studies had suggested an erythema action spectra for 8-MOP of 360 nm (Pathak, Kramer & Fitzpatrick, 1974).

For chlorpromazine, the action spectra has been variously reported either above or below 320 nm (Epstein, Blunstein & Peterson, 1957; Epstein, 1968). There is an interesting lack of correlation between the absorption spectra for chlorpromazine (350 nm) and its erythema action spectra in animals and man. It may be that this is due to the modulating effects of the skin on selected UV irradiation.

With coal tars, the erythema action spectra have ranged between 340 nm and 400 nm (Crow *et al.*, 1961; Everett & Miller, 1961). An interesting clinical point about coal tar phototoxicity is that when coal tars and UVA are used in combination they are effective phototoxic agents but are poorly effective in the therapy of psoriasis. Most psoriasis treatment regimens utilize predominantly UVB irradiation with coal tar and may, therefore, not act therapeutically via phototoxic mechanisms (Lowe *et al.*, 1983).

The action spectra for erythema production with other substances also varies widely with the substance. For example, tetracycline produces an action spectra above 320 nm while certain sulphonamides have action spectra in the 370–400 nm range. The action spectra for most phototoxic substances are predominantly in the UVA range. This has practical implications for phototesting of potential phototoxic agents and a broad band UVA source should usually be utilized. Various UV sources can be used that have this criteria, including Hot-Quartz UV lamps with window glass or mylar filters. A bank of predominantly UVA fluorescent tubes is useful; however, it should be noted that these tubes also contain contaminating UVB (290–320 nm). Hence, if they are to generate a relatively pure UVA spectrum, they should be used with appropriate mylar or window glass filter. In addition, narrow band 365 nm light may be obtained using a Woods Light. Clearly, monochromatic sources are necessary where more detailed clinical investigation is required.

As an alternative to window glass or mylar filter, appropriate cut-off filters have been employed, depending on the UV spectrum desired for phototesting. A good review of UV equipment and techniques utilized in phototesting is given by Johnson and McKinsey (1982).

## *IN VIVO* ANIMAL STUDIES OF PHOTOTOXICITY

A variety of animals have been used, most notably the hairless mouse (Forbes, Davis & Urbach, 1976). Guinea-pigs have also been used extensively to study a variety of photosensitizing agents: Korhnauser *et al.* (1982) showed that there is a reasonably good correlation between guinea-pig responses and human phototoxicity responses for a number of different psoralens. The guinea-pig model has also been used to study the photosensitizing ability of different coal tar products (Hebborn & Cram, 1977).

In the hairless mouse, one can measure the dorsal skin thickness using vernier skin fold calipers. In this way the degree of dermal oedema induced by phototoxic agents is measured readily and is not subject to the problems of visual assessment of erythema in rodent skin. In these latter studies we also examined the ability of psoralens and UVA to induce the enzyme ornithine decarboxylase (ODC). ODC is the rate-limiting enzyme in the biosynthesis of polyamines (Tabor, 1976). The polyamines appear to be important precursors of cellular hyperplasia, while ODC in itself appears to be a useful marker of cellular damage. Noteworthy here is that psoralens that were phototoxic, i.e. 8-MOP and 5-methoxypsoralen (5-MOP), and UVA significantly induced ODC and oedema in the hairless mouse; but a non-phototoxic psoralen, i.e. 3-carbethoxypsoralen (3-CP) and UVA failed to induce either (Lowe *et al.*, 1984). In these studies we also examined the ability of the different psoralens and UVA to inhibit the rate of thymidine incorporation into the epidermis. 8-MOP and 5-MOP produced a profound suppression of thymidine incorporation 4 h following psoralen and UVA irradiation. However, 3-CP produced no significant change in DNA synthesis. Thus, it would seem that for those phototoxic substances that have an effect on cellular DNA, the thymidine incorporation assays *in vivo* are useful.

A thymidine incorporation assay has also proved valuable in assessing the suppressive activity of different coal tar products on epidermal DNA synthesis in mouse skin with and without UV irradiation (Lowe, Breeding & Wortzman, 1982).

Longer-term studies of phototoxic effects of psoralens on the hairless mouse include the ability of psoralens and UVA to produce skin malignancy in these animals. A variety of researchers have investigated the potential for photocarcinogenicity of psoralens in the mouse (O'Neil & Griffin, 1957). These studies tend to be time-consuming and costly, but it may be possible to utilize the more acute phototoxicity markers of skin erythema and oedema, as well as certain cellular events such as alteration of thymidine incorporation, ODC activity and sunburn cell production to predict the potential for phototoxic carcinogenesis.

A further system that has been studied in animals is the ability of different phototoxic agents to deplete glutathione. Glutathione is the principal endogenous pre-radical scavenging thiol in the skin. It is known that substances such as sulphonamides (Schothorst, Suurmond & Delustra, 1979) and psoralens (Wheeler *et al.*, 1985) followed by UVA will deplete cutaneous glutathione. In recent studies we examined the ability of 8-MOP and UVA to deplete cutaneous glutathione. The glutathione was depleted in a dose-dependent fashion and correlated well with the phototoxic ability of the psoralen as measured by oedema and erythema (Wheeler *et al.*, 1985). ODC was also included in these experiments. Precise mechanisms of glutathione depletion are currently being investigated and seem not to involve the oxidation of glutathione to oxidize glutathione. It is possible that depletion of glutathione is an important mechanism for cellular toxicity as has also been suggested for certain hepatotoxic drugs.

## *IN VITRO* ASSAYS FOR PHOTOTOXICITY

A variety of assays have been used to predict phototoxic potential which avoid the use of animals and humans. One can evaluate the toxic effects of chlopromazine and ultraviolet light utilizing *Escherichia coli* (Matsuo *et al.*, 1985).

Other bacterial systems being used include the ability of different psoralens to induce prophage. Prophage induction is thought to occur as a result of the SOS response to DNA damage by ultraviolet light and photo-mutagenic agents in some bacteria. The cytotoxicity and ability to induce the expression of prophage in *Salmonella typhimurium* was examined for different furocoumarins, 8-MOP, 5-MOP, 3-Cp and 5-methylisopsoralen (5-MI) in combination with UVA irradiation. We found utilizing this assay that 8-MOP, 5-MOP and 5-MI were potent inducers of prophage expression (Connor, Wheeler & Lowe, 1983). Conversely, 3-CP was a very poor inducer of prophage. In subsequent animal phototoxicity studies, 3-CP proved non-phototoxic while the other psoralens mentioned above were significantly phototoxic. Therefore, it seems that the ability to induce expression of prophage in *Salmonella typhimurium* represents a rapid and useful screen for the potential phototoxicty and carcinogenicity of novel psoralens (Connor *et al.*, 1983).

Another bacterial assay has been reported recently as being useful for studying the phototoxicity of benzothidiazine diuretics. Fujitah and Matsuo (1985) used bacterial phage lambda to study the ability of these different agents to inactivate the phage. *Escherichia coli* was used as the indicator bacteria. Hence, a variety of bacterial systems appear to predict phototoxicity and further work is needed to compare and contrast these results with phototoxicity reactions in animal and man.

Another *in vitro* assay that has been used widely over the last two decades takes advantage of the ability of phototoxic substances to damage the pathogenic yeast *Candida albicans* (Daniels, 1965). This test was found to predict clinical phototoxicity of compounds such as sulphanilamide and demeclocycline reliably. Further refinements of this assay have utilized a non-pathogenic yeast *Candida utilus* (Kagen & Gabriel, 1980). These *in vitro* tests are simple and easy to use and thus may be used as a first-line screening test, together with animal phototoxicity studies.

A mammalian cell culture system used peripheral blood mononuclear cells to compare the phototoxic potential of various chemicals (Morrison *et al.*, 1982).

## PHOTOTOXICITY REACTIONS IN MAN

Phototoxic reactions in man should theoretically occur in the whole population, provided that sufficient amount of drug is present in the skin and that the appropriate amount and wavelengths of ultraviolet light are present. In clinical practice, however, wide variations in phototoxicity occur which depend on a number of factors, including the varied pharmacokinetics of systemically administered drugs as well as different achievable drug levels within the skin. For topically-applied agents, the amount of drug in the skin is dependent on the percutaneous absorption of the drug and in a particular vehicle. The metabolism of phototoxic agents will also alter the phototoxicity of these agents as well as determine the excitation wavelengths required to produce phototoxicity. As regards topical agents, the selection of drug vehicle for the study of phototoxic substances is of critical importance (Kaidbey & Kligman, 1974).

Individual skin type is another key factor in determining the minimal levels of phototoxicity often known as the minimal phototoxic dose (MPD) for an individual. Skin melanin content and pigmentary responsiveness have been utilized as a means of predicting that MPD may occur. In general, the darker the skin type, the higher the MPD for a given substance. For example, in clinical practice, phototoxic reactions are much rarer in black skin than in caucasian skin.

Frosch and Wissening (1982) reported on the use of skin irritation using different chemical irritants and the ability of a UV-induced minimal erythema dose (MED) to determine hyper-irritable skin. This approach may be useful to determine the skin type of an individual prior to phototoxic testing in an attempt to estimate the MPD, which may be utilized to avoid unnecessary phototoxic skin burns.

Monochromatic skin irradiation has been utilized as a means of identifying the action spectra for a variety of photosensitivity diseases and phototoxic substances (Cripps, 1981). In this review, Cripps reports the erythema action spectra using a prism grating monochromater in skin diseases where endogenous photosensitivity is a problem. Such diseases included xeroderma pigmentosum, lupus erythematosis, polymorphous light eruption, solar urticaria, actinic reticuloid and persistent light reactors. Action spectra for psoralen phototoxicity were discussed.

We consider that the routine skin testing of cosmetics and new topical agents should include phototoxicity testing. Such routine screening will only be predictive if the vehicle permits sufficient skin penetrance of the potential photosensitizer. The appropriate selection of ultraviolet wavelength range is important. A broad spectrum ultraviolet source that emits wavelengths throughout the UVA range is important to increase the likelihood of producing a phototoxic reaction. In addition, the individual should be tested for both their MED and MPD.

We have recently utilized the ability of topical psoralens to produce a dose-dependent MPD to evaluate UVA sunscreening agents (Lowe *et al.*, unpublished observations). We found this to be an extremely reliable method of evaluating the photoprotective effects of a UVA sunscreening agent. This may have practical clinical relevance because some patients are using drugs that have a high risk of phototoxicity; for example, systemic psoralens and thiazide diuretics. It should be noted that some substances seem to have both photo-allergic as well as phototoxic potential, such as the phenothiazine derivatives. Other substances are able to produce immediate as well as delayed phototoxicity responses, e.g. the non-steroidol anti-inflammatory agent, benoxaprofen. Noteworthy here is that benoxaprofen seemed to have a wide erythema action spectra that extends into UVB spectrum in addition to the UVA (Ferguson *et al.*, 1982).

The use of human volunteers for evaluation of the phototoxicity potential of cosmetics and topical drugs is of paramount importance prior to the release of these substances into the general population. These human-testing procedures should be undertaken only after the appropriate *in vitro* and animal tests have been performed.

The use of predictable phototoxic agents such as 8-MOP is valuable as a means of ranking the phototoxic potential of another phototoxic substance. In addition, the use of predictable phototoxic substances such as 8-MOP can help evaluate potential UVA sunscreening agents. It is probably inadvisable at this stage to allow psoralen-containing sunscreens to be marketed due to the potential danger of long-term photocarcinogenicity of these drugs. At this time, it is not known whether the potential photoprotective properties of a psoralen-induced tan outweigh the long-term risk.

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# The 'angry back': a new concept or old confusion?

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The term 'angry back' was created 10 years ago by Mitchell in an extraordinary paper. No data were presented but proclamations were plentiful, culminating in the provocative declaration that 'eczema creates eczema' (Mitchell, 1975). The term caught on instantly and is now entrenched in the literature as dermatological dogma. We regard the 'angry back' as a controversial notion whose appeal lies largely in its neat ability to explain troublesome results in patch testing. Additionally, the term is semantically slick and oversimplifies a complex situation. A thorough critique of the 'angry back' syndrome will be presented elsewhere. Our purpose in this paper is to register scepticism, to urge others to review the issues more critically, and to present some experimental data which contravene the concept.

To bite the bone of contention directly, one can focus on the controversy by quoting Mitchell who explicitly defined the 'angry back' in his 1977 paper (Mitchell, 1977). 'A single strong positive patch test reaction creates an angry back which is hyper-reactive to other patch test applications. Concomitant false-positive reactions are thus frequently obtained.'

Mitchell's chief concern was with the problem of false-positive patch tests which arise, he avers, because of the practice of applying multiple patches at the same time. His basic observation is that many positive reactions will be lost if the test substances are reapplied later, *one at a time*. Among ninety 1+ reactions to a battery of standard allergens, fully 42% were lost upon single retesting one week later (Mitchell, 1977). He concluded that 'in the presence of one strongly positive patch test, all other positive reactions must be viewed with suspicion. No credence can be given to any other concomitant positive reactions. The suspected chemicals must be tested sequentially one at of time, otherwise it cannot be assumed that the reactions were truly allergic.'

This revelation was received warmly by international authorities. Maibach expanded the concept by renaming it the 'excited skin syndrome', acknowledging that the entire skin, not just that of the back, could be rendered hyper-active by one strongly-positive reaction (Maibach, 1981). Mitchell and Maibach (1982) then presented a comprehensive exposition of the doctrine. They warned ominously that those who performed only batteries of patch tests would regret it. False-positives were very serious and could lead to false legal settlements and unwarranted job changes.

Other centres soon supplied confirmatory reports. The International Contact Dermatitis Group re-evaluated 56 patients with two or more positive reactions (Maibach *et al.*, 1982). Forty-three percent were lost when retested sequentially. Bruynzeel and his collaborators are enthusiastic proponents. His doctoral thesis summarizes extensive studies conducted in the

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Netherlands (Bruynzeel, 1983a). In one report on 61 patients retested after 3 weeks, 44% positives were lost (Bruynzeel *et al.*, 1983c). This percentage turns up with astonishing frequency, exceeded only by the 60% loss rate of Luderschmidt *et al.* (1982). According to these stalwarts, even 4+ reactions should be repeated one at a time.

Grosshans and Foussereau (1983) also stand in admiration, calling sequential testing the 'bonne methodologie' which has solved one of the major complications of patch testing. Fisher (1982) recalls that Max Jessner taught that a strongly-positive patch test could induce other false-positive reactions. Jessner called this the 'crazy back', anticipating Mitchell by 40 years. The only critics of the 'angry back' concept are Bandmann and Agathos (1981a). They retested 1040 positives in 40 patients, and only 8·6% were lost.

## METHODS AND RESULTS

We singled out for experimental analysis the central element in the 'angry back' concept, namely, that one strongly-positive patch test can induce a state of generalized hyper-irritability. Our subjects were healthy student volunteers who had been artificially sensitized to a variety of allegens by the maximization procedure. Many were also strongly allergic to Rhus oleoresin, having experienced poison ivy dermatitis repeatedly. We used occlusive exposures throughout, generally loading the allergens and irritants into 12 mm aluminium Duhring Chambers, holding about 0·2 ml of test substance. Our aim was to study semi-quantitatively interactions among irritants and allergens.

### *Study 1*

The subjects were eight males who had a strongly vesicular, spreading reaction (3+ to 4+) to a 1:50 ethanolic extract of Rhus oleoresin by a 24 h chamber exposure.

TABLE 1. Four strongly-positive Rhus reactions do not enhance sodium lauryl sulphate irritancy

| | SLS alone* | | SLS + Rhus | |
|---|---|---|---|---|
| Subject | 0·5% | 2·0% | 0·5% | 2·0% |
| 1 | 1 | 1 | 0 | 1 |
| 2 | 0 | 2 | 1 | 2 |
| 3 | 0 | 2 | 0 | 1 |
| 4 | 1 | 1 | 1 | 2 |
| 5 | 1 | 1 | 1 | 2 |
| 6 | 1 | 2 | 0 | 1 |
| 7 | 0 | 2 | 1 | 2 |
| 8 | 0 | 1 | 0 | 1 |
| Mean | 0·5 | 1·5 | 0·5 | 1·5 |

* 1 = mild redness; 2 = moderate redness, slight oedema; 3 = strong redness, swollen, tender.

First, the intensity of the reactions to 0·5% and 2·5% aqueous sodium lauryl sulphate (SLS) was established by a 24 h exposure on the upper back, with readings 3 h later.

Three weeks later, four 1:50 Rhus chambers were applied for 24 h on one side of the upper back, at the corners of a 6 inch square. Simultaneously, two chambers containing 0·5% and 2·5% SLS were placed centrally, equidistantly from the corners. Thus, the irritant patches were surrounded by Rhus patches. Table 1 shows that the SLS reactions at 24 h were the same in the presence and absence of very strong reactions to Rhus oleoresin. Thus, four strongly-positive allergic reactions did not elevate the skin's reactivity to a classic irritant, the anionic surfactant, SLS.

*Study 2*

We recruited nine females who gave a strongly vesicular reaction to 1:50 Rhus oleoresin. They were also natively sensitized to nickel and gave weak to moderately strong positive allergic reactions to 5% aqueous nickel sulphate.

First, the intensity of a 24 h nickel sulphate patch test was determined. Four to six weeks later, four 1:50 Rhus oleoresin patches were applied at the corners of a six inch square on one side of the back. Simultaneously, a 5% nickel sulphate patch was placed in the centre of the square. Table 2 shows that the original nickel sulphate reactions were not intensified by four concomitant Rhus patches. This result also held true for readings made at 48 h.

*Study 3*

Can strong reactions to the potent allergen, dinitrochlorobenzene (DNCB), enhance the intensity of irritant reactions to anionic and cationic surfactants?

We sensitized male volunteers to DNCB by applying 0·5% DNCB in petrolatum for 24 h to the forearm. Six subjects were selected who showed spontaneous flares at the exposure site 7–10 days later.

TABLE 2. Four strongly-positive Rhus reactions do not enhance allergic reactions to 5% nickel sulphate

| | Single exposure* | | Concomitant exposures | |
|---|---|---|---|---|
| Subject | Nickel sulphate | Rhus | Nickel sulphate | Rhus |
| 1 | 1 | 2 | 1 | 3 |
| 2 | 2 | 3 | 1 | 3 |
| 3 | 2 | 3 | 2 | 3 |
| 4 | 1 | 3 | 1 | 3 |
| 5 | 1 | 2 | 1 | 3 |
| 6 | 1 | 3 | 1 | 3 |
| 7 | 2 | 3 | 2 | 3 |
| 8 | 1 | 2 | 2 | 2 |
| 9 | 1 | 3 | 1 | 3 |
| Mean | 1·3 | 2·5 | 1·3 | 3·0 |

* 1 = moderate redness, slight oedema; 2 = strongly red, palpable induration; 3 = intense redness, papulo-vesicles, spreading.

TABLE 3. Extremely strong allergic reactions to DNCB do not intensify the inflammatory responses to sodium lauryl sulphate (SLS) or benzalkonium chloride (BC)

| | 1% SLS reactions | | 0·5% BC reactions | |
|---|---|---|---|---|
| Subject | Alone | SLS+DNCB | Alone | BC+DNCB |
| 1 | 1 | 0 | 1 | 1 |
| 2 | 1 | 2 | 2 | 2 |
| 3 | 1 | 1 | 2 | 2 |
| 4 | 2 | 2 | 1 | 1 |
| 5 | 1 | 1 | 2 | 2 |
| 6 | 1 | 1 | 2 | 2 |
| Mean | 1·2 | 1·2 | 1 7 | 1·7 |

First, the intensity of the reactions to 1% aqueous SLS and 0·5% benzalkonium chloride (BC) in 24 h chamber exposures on the back was determined. Then, 3 to 6 weeks later, four chambers containing 0·1% DNCB in petrolatum were applied for 24 h at the corners of a six inch square. Simultaneously, the subjects were re-exposed to SLS and BC within the centre of the square. Table 3 shows that intense 4+, spreading reactions to DNCB (not recorded) did not intensify inflammatory responses to either irritant. The backs did not become 'angry' under the conditions of this experiment.

*Study 4*

Can spill-over account for enhanced reactivity when multiple patches are applied?

Mitchell (1975) suggested that false-positive reactions were more likely to occur in the immediate neighbourhood of a strongly-positive allergic patch. He thought lymphatic drainage might account for this non-specific exaltation of closely-spaced patches. The motion of 'spill-over' has scarcely ever been doubted.

Bruynzel (1983a) found evidence for the phenomenon, noting that false-positive reactions were likely to be found in proximity to strong positive allergic reactions. Moreover, he and his co-workers designed a study which proved the validity of the spill-over phenomenon (Bruynzeel *et al.*, 1983b). They placed marginally irritating concentrations of SLS at various distances from positive allergic reactions and could show, clinically and histologically, that the irritant responses were greater in the neighbourhood of alergic patches.

Fisher (1982) endorses the spill-over concept as a source of false positives. Bjornberg (1968) also thought that a single allergic reaction might intensify nearby reactions.

We investigated spill-over using various configurations of allergic and irritant chamber patches, near and far from each other. Our results were very similar to those of Bandmann who placed strong irritants and allergens in a ring (cocarde) around a central allergic patch. He failed to obtain any enhancement from proximity (Bandmann & Agathos, 1981b).

We collected a panel of volunteers whom we had sensitized to both cinnamic alcohol and isoeugenol by the maximization procedure. These persons reacted on the arms in varying intensity to 48 h exposures. First, the two allergens were applied at 1% concentrations in petrolatum on opposite arms for 48 h. Two days later the exposures were repeated on the same

TABLE 4. Allergic reactions to isoeugenol and cinnamic alcohol are the same whether placed close together on one arm or on separate arms

| | Isoeugenol | | Cinnamic alcohol | |
|---|---|---|---|---|
| Subject | Same arm | Opposite arms | Same arm | Opposite arms |
| 1 | 2 | 2 | 1 | 1 |
| 2 | 3 | 3 | 2 | 2 |
| 3 | 1 | 1 | 0 | 0 |
| 4 | 0 | 0 | 1 | 2 |
| 5 | 1 | 1 | 1 | 1 |
| 6 | 3 | 2 | 2 | 2 |
| 7 | 2 | 2 | 0 | 0 |
| Mean | 1 7 | 1·6 | 1 0 | 1·1 |

arm, separated by a distance of 2 cm. Table 4 shows that the reactions were the same whether the allergens were in close proximity or on opposite arms. No evidence of a spill-over was found. In our view, spill-over reflects sloppy technique in which the test chemical is not confined sufficiently to the test site, thereby contaminating nearby skin.

## DISCUSSION

We have been unable to show experimentally that a single positive patch test creates an 'angry back'. The latter is a convenient explanation for false-positive reactions, but it does not rest on a solid experimental foundation. Belief in Mitchell's thesis derives from the non-repeatability of positive tests. We submit that there are a number of alternative explanations for false-positive patch tests, not the least of which is the inherent variability of patch testing, a truly primitive diagnostic procedure. We cannot go into the numerous limitations. We certainly do not believe that anywhere near 40% of the reactions which turn up on routine testing will be lost.

Nonetheless, there is incontrovertible evidence that the skin may become hyper-irritable to substances which are normally harmless. In fact, the concept of hyper-reactive skin was established long ago and its reality has been appreciated by clinicians for more than half a century. One of the first demonstrations was by the distinguished von Zumbusch, in 1921. He found that patients with eczema reacted to multiple materials which were non-irritating to normals (von Zumbusch, 1921). Later, Bloch (1924) introduced the term 'status eczematicus' for the same phenomenon. Geiger's report in 1929 was one of many soon to follow which warned about non-specific reactions in eczema patients. He found that 90% of these reacted to 60% turpentine in contrast to only 6% in normals (Geiger, 1929). An up-to-date review of the literature on hyper-irritable skin has been prepared by Roper and Jones (1982).

In 1955, Wilson brought into focus the vexing problem of differentiating irritant from allergic reactions (Wilson, 1955). He realized that many patients with eczema could tolerate substances to which they were patch test positive. What was the relevancy of positive patches if these substances did not have to be avoided? Wilson anticipated Mitchell by showing that some of the positives were lost upon retesting. His seminal observation was that the skin was hyper-irritable during the stage of active dermatitis. After clearing, the skin lost its hyper-irritability. Bjornberg (1968) demonstrated this in his classic study of chronic hand dermatitis.

Unfortunately, confusions creep in when multiple terminologies come into use. Hyper-irritable skin syndromes have been classified under the following titles: (i) status eczematicus, (ii) hyper-reactive skin, (iii) conditioned irritability, (iv) polyvalent sensitization, and, most recently, (v) the excited skin syndrome, known as the 'angry back'. Semantic diversities of this kind are bound to lead to nosological confusions. Thus, we find authors who lump other syndromes inappropriately with the crazy back (Roper & Jones, 1982). An example of this is auto-eczematization, a term invented by Whitfield (1921), and later elaborated by Haxthausen (1955) and Young (1958) under the title of autosensitization dermatitis. While autosensitization develops against a background of hyper-irritable skin, it is nevertheless a distinctive, separate syndrome. It begins as a localized persistent dermatitis, frequently statis dermatitis. Aggravation of the primary lesion by trauma, chemical irritation, contact sensitization or overtreatment incites a papulo-vesiculo eruption in other part of the body, often in a characteristic pattern. The cause of such sudden dissemination is disputed.

Conditioned irritability, status eczematicus, hyper-irritable skin, the 'angry back' etc. all refer to a general state in which the skin's reactivity to allergens and irritants has become transiently elevated. The many reports on this subject share a common feature. The subjects are patients who come to a skin clinic with a long-lasting dermatitis. Stasis dermatitis is probably the most frequent incitant. A single episode such as acute poison ivy dermatitis is insufficient and may even result in temporary unreactivity (anergy) (Kligman, 1958).

Patch testing with multiple potential allergens, 20 or more at a time, is dictated by practical necessity. Moreover, the testing is usually performed while the patient's dermatitis is still active. Also, many of the test substances are marginal irritants. This is the crux of the matter. We submit that it is the pre-existing dermatitis and not one or more strongly-positive patch tests which makes the skin hyper-irritable. When the patches are repeated at a later time, the dermatitis may have started to fade. The low percentage of lost positives in Bandmann's series is very likely due to his having waited until the dermatitis cleared before retesting (Bandmann & Agathos, 1981a). Bruynzeel, too, noted that the percentage of lost reactions was lowest in the group whose dermatitis was still active (Bruynzeel *et al.*, 1983c). We have no way of knowing the original level of hyper-excitability. This will certainly be very variable depending on the duration, intensity, cause and extent of the eczema. In any case, the necessity of repeating the patch tests one at a time has not been demonstrated. The sceptic will ask what would have happened had the tests been repeated two of three at a time, with and without retesting with a strongly positive allergic reaction?

One can fault the 'angry back' concept on a number of accounts, but it would be tedious to do so here. To mention one difficulty, what constitutes a weak, 1 + reaction? Readers of patch tests use different criteria of positivity. More positives will be lost when the threshold is merely erythema than when there are other signs, *viz* induration-elevation, spreading, itching etc. We are hampered by insufficient information. Were the patients hospitalized or out-patients? A breakdown according to occupation is an important variable. If the clinic draws upon workers in the clinical industry, exposed to potent allergens such as chromates and epoxy resins, the magnitude of false-positives may be very different from housewives and executives. The data base is flimsy.

Finally, we must concede that our negative results do not, by themselves, nullify Mitchell's version of the 'angry back'. Our subjects were normals and did not have hyper-irritable skin at the time of testing. We think that the 'angry back' has confounded the problem of hyper-irritable skin. A new name does not bring new light. The term is a catchy cliché, a slick label, to help the troubled patch tester out of one of the more frequent diagnostic difficulties inherent in patch testing. Perhaps we need an equally creative term to deal with an equally important diagnostic problem, that is, the false-negative patch test. We suggest the 'silent back syndrome'.

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## DISCUSSION SUMMARY

The following points were raised:

(1) Dr Kligman could not demonstrate the 'angry back' with DNCB patch tests but he clearly demonstrated an 'excited skin state' with 25% kerosene—Mitchell's manifesto of 1975 was a new description of a known phenomenon described in 1929 by Geiger: a patient with acute chronic dermatosis is more susceptible to give false-positive reactions to patch tests.

The disagreement between Dr Kligman and Dr Maibach lies in the difference in the percentage of false-positive tests reported (8% against 40%).

(2) Any human positive patch test should be repeated for confirmation.

(3) In the maximization test on guinea-pig skin, one should perform a further patch test on the same animal several months later in order to confirm a strong-positive reaction and to infirm a weak one.

(4) On the kerosene-irritated patients we can observe an increase of serum neutrophils from 20 000 to 30 000/mm$^3$, as well as an increase in basophils and eosinophils, which reflects a systemic action.

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# Effects of topical corticosteroids on human dermis

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The development and employment of effective topical glucocorticosteroids (steroids) have revolutionized dermatological therapy. Steroid applications have been very successful in controlling inflammatory reactions of the skin and are one of the most widely used class of compounds in the dermatologist's armamentarium. The original active topical steroid was hydrocortisone (Sulzberger & Witten, 1952). After the introduction of this, numerous structural modifications were made which improved efficacy. Inherent activity of hydrocortisone was enhanced by insertion of an extra double bond giving rise to prednisolone and prednisone. Addition of 6 alpha fluro and/or 9 alpha fluro derivatives yielded triamcinolone, betamethasone and fluocoinoide (Robertson & Maibach, 1982). A major modification was the discovery that acetonide derivatives of fluorinated steroids markedly enhanced the biological activity (McKenzie & Atkinson, 1964). Unfortunately, some of these more clinically beneficial preparations are associated with unwanted adverse changes in skin, such as telangiectasia, epidermal and dermal atrophy and striae, as well as some systemic side-effects.

Perhaps the most commonly encountered, serious, and troublestome side-effect is steroid-induced atrophy and the inevitable rebound phenomenom after steroid discontinuation. Telangiectasia, thinning, shininess with loss of skin markings, and increased transparency, are all part of the constellation of clinical signs that dermatologists observe (Epstein, Epstein & Epstein, 1963; Schopf, 1975; Sneddon, 1976). Unravelling the mechanisms of these changes has been difficult. It is well known that steroids depress both mitotic activity and DNA synthesis in epidermal cells, which results in epidermal thining (Fisher & Maibach, 1971; Marks *et al.*, 1971). Studies in our laboratory using a potent steroid (clobetasol-13-propionate) under occlusion for 6 weeks resulted in a 59% decrease in viable epidermal thickness, flattening of the dermal/epidermal junction, and virtual deletion of the stratum corneum (Lehmann *et al.*, 1983). Similar morphological findings have been reported by other investigators (Stevanovic, 1972; Wilson-Jones, 1976; Winter & Burton, 1976; Kirby & Munro, 1976; Jablonska, Groniowska & Dabrowski, 1979). While agreement generally exists concerning epidermal changes, investigators have divergent views on the primary alterations in the dermal matrix after prolonged steroid usage. All agree that alterations in the synthetic capacity of fibroblasts is the primary result of steroid application. Differences exist, however, as to the extent that collagen and/or elastin is altered. Some investigators observed elastic fibre degeneration, whereas collagen was unaltered (Stevanovic, 1972), while others reported that collagen was primarily affected (Groniowska *et*

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*al.*, 1976; Jablonska *et al.*, 1979). These assessments of fibrous alterations relied solely on conventional histological and transmission electron microscopic (TEM) visualizations. Unless dramatic changes occur to either collagen or elastin, these methods provide little insight into overall dermal reorganization. Over the past several years, our laboratory has not only used previously described methods but has also approached this problem using scanning electron microscopy in conjunction with special preparative procedures (Lehmann *et al.*, 1983). During the course of these investigations we observed that not only was the fibroblast effected, but that steroids had a major effect on dermal mast cells (Lehmann *et al.*, 1983; Lavker & Schechter, 1985). We now present a review of our recent findings and comment on their clinical significance.

To stimulate 'long-term' steroid usage typical of many treatment regimes and to maximize the delivery of the steroid, cutaneous atrophy was induced on the forearms of human volunteers by continuous occlusive application of steroids for 6 weeks. Cutaneous changes were followed *ad seriatum* by light, scanning and transmission electron microscopy. In addition, histamine and serine proteinase levels were determined at various time intervals (Lehmann *et al.*, 1983; Lavker & Schechter, 1985). Subsequent studies on patients with urticaria pigmentosum involved treatment with either intralesional injections of steroid, or by topical application of steroids under partial occlusion, followed by clinical, morphological and biochemical evaluations of affected sites during and post-treatment (Barton *et al.*, 1985).

## EFFECTS OF SKIN DURING STEROID TREATMENT

Epidermal and dermal atrophy were clearly evident after 3 weeks of treatment to normal skin. At this time, the epidermis was appreciably thinned and viable epidermal thickness was reduced 42%. A marked compaction of the papillary dermis was noted at this time and rearrangement of both the collagen and elastic fibre networks was apparent. By 6 weeks, these changes were magnified and both the papillary and reticular dermis were involved. Profound changes were seen in the fine structure of the fibroblast, although their number did not appear significantly reduced. Normally, fibroblasts are elongated cells with abundant cytoplasm containing prominent profiles of rough endoplasmic reticulum filled with flocculent material. After 6 weeks, they were smaller, appeared more ovoid, and the cytoplasm was markedly reduced so that the nucleus made up most of the cell mass. This synthetically quiescent appearance may have been responsible for loss of Hale's stainable material which suggests some elimination of the ground substance. This is consistent with reports demonstrating that steroids inhibit the synthesis of collagen and glycosaminoglycans (Cohen, Diegelmann & Johnson, 1977; Pinnell, 1982; Ponec *et al.*, 1979; Priestly, 1978; Russel, Russel & Trupin, 1978; Sarni & Hopsu-Havu, 1976, 1978; Uitto, Teir & Mutakillo, 1972). Ground substance occupies the spaces between collagen bundles and elastic fibres. The decrease in ground substance may explain the resulting spatial contraction and compression of both collagen and elastic fibres. When viewed in the SEM, the fibres straightened and became parallel to each other, completely altering the 3-dimensional architecture. However, no gross differences in amounts of collagen and elastin, and/or in their fine structure were noted.

Concomitantly, the mast cell population was also affected. During the initial phase of atrophy (first 3 weeks) mast cell morphology and tissue histamine levels were virtually unchanged, despite the marked epidermal and dermal alterations. However, after 3 weeks, histamine levels fell, decreasing to less than 85% of pre-treatment levels by 6 weeks. The loss of mast cell constituents was demonstrated by another biochemical criteria. Human skin has been shown to contain a trypsin-like and chymotrypsin-like proteinase, which evidence indicates are both mast cell constituents (Schechter *et al.*, 1983; Fraki & Hopsu-Havu, 1975; Schwartz, Lewis & Austen, 1981). After 6 weeks, less than 10% of these proteinases were detected in skin. TEM examination of 6 week biopsies indicated that the reduced levels of histamine and proteinases were associated with the depletion of mast cells as evidenced by the inability to identify any cells representative of mast cells and the marked acellularity around the vasculature where mast cells are usually found in abundance.

In some biopsies taken after 3 weeks of treatment, a small population of mast cells was observed that appeared to be degenerating. They were rounded, without microvillus extensions and featured a cytoplasm containing clumped chromatin-like material, disrupted mitochondria, vacuoles filled with flocculent material and some intact granules. Appearance of these degenerating mast cells, the subsequent decline in histamine and proteinase levels following this time point, and the inability to detect mast cells after 6 weeks, suggests that steroids may have been toxic to mast cells (Lavker & Schechter, 1985).

An intriguing aspect of the steroid-mast cell relationship revolves around different degrees of histamine depletion with different steroids. After 6 weeks of topical treatment, clobetasol-13-proprionate and fluocinonide resulted in an 85% decrease in histamine levels, whereas betamethasone valerate produced only a 66% reduction in histamine levels (Lavker & Schechter, 1985). This suggests a possible relationship between mast cell disappearance and steroid structure. It will be of interest to see whether the disappearance of mast cells is correlated with different steroid structural modifications.

## RECOVERY OF SKIN FROM STEROID TREATMENT

A general consequence of long-term topical steroid usage is that, after treatment is stopped, a rebound phenomenon develops which takes the form of a dermatitis with intense redness, scaling, crusting, and even pustulation (Sneddon, 1969; Burry, 1973; Weber, 1976; Calnan, 1976; Kligman & Frosch, 1979). Epidermal hyperplasia which typifies the rebound phenomena reflects both a repair of the lost barrier due to thinning of the stratum corneum during atrophy, and repair of the atrophic, suppressed epidermis after alleviation of an antiproliferative agent. In the post-steroid dermis, hyperactivity of fibroblasts was evidenced by widely dilated endoplasmic reticulum filled with flocculent material. This correlated with a rapid increase in glycosaminoglycans, reaching normal levels in 14 days. Overall cutaneous restitution is dramatically rapid; the epidermis and dermis appear practically normal by 14 days. The rapid changes seen in basal epidermal cell and fibroblast fine structure immediately post-steroid (0–2 days) signifies a rapid clearance of the steroid from the skin (Zheng *et al.*, 1984).

In contrast to the above reparative events, return of mast cells in the skin is significantly delayed. No significant increase in histamine levels was observed until after 14 days of restitution. Following this 2–3 week delay, histamine levels began to rise, and by 3 months they were returned to near normal levels. Detailed TEM observations after steroid discontinuation were consistent with the histamine findings. Four to seven days post-steroid, the dermis was quite cellular, containing numerous lymphocytes, macrophages, and fibroblasts; however, mast cells were not in evidence. At 14 days, mature mast cells were still not detectable, but some cells containing sparse amounts of mast cell-like granules, as well as microvillus surface extensions, were noted (Lavker & Schechter, 1985).

The precise origin of the mast cell in man is unknown. In studies on murine species it is becoming clear that connective tissue mast cells originate from the haemopoietic stem cells of the bone marrow (Kitamaura *et al.*, 1981; Metcalf & Moore, 1971; Hatanaka, Kitamura & Nishimune, 1978), and that precursors to mature skin mast cells may be found in the circulatory system (Zucker-Franklin *et al.*, 1981; Sonoda, Ohno & Kitamura, 1982), and may be fixed in the skin itself (Matsuda *et al.*, 1981). Indirect evidence suggests that progenitors from the blood are not granulated. The likely explanation for the delayed rise in histamine is that steroids result in the loss of mast cells entirely from skin, and that 2–3 weeks is the time needed before they begin repopulation.

It could be argued that mast cells were always present in a non-granulated form, and that they were impossible to detect morphologically. Failure of histamine levels to rise for a minimum of 2–3 weeks after steroid was discontinued would then represent the minimum time needed for viable mast cells to respond to removal of inhibitory factors. This seems an unreasonably long period when compared to the recovery time of keratinocytes, melanocytes and fibroblasts. These cells were severely attenuated during steroid treatment, but displayed rapid recovery within 2 days following steroid discontinuation (Zheng *et al.*, 1984). Whether the disappearance of mast cells is related to a direct effect of steroids or secondarily to the atrophy process is still unknown.

## CLINICAL APPLICATIONS OF STEROID-INDUCED MAST CELL DEPLETION

We used the mast cell depleting effects of steroids to treat urticaria pigmentosum (UP), a rare skin disease characterized by increased numbers of normal-appearing mast cells in skin. Some patients were treated by intralesional injection of steroids and others by topical application under occlusion to a limited area 8 h daily for 6 weeks. Intralesional injections resulted in resolution of the urticaria pigmentosum lesions, as well as removal of mast cells as judged by TEM and tissue histamine content. Injected sites remained dramatically improved for as long as one year after treatment (Barton *et al.*, 1985). This is not surprising as intradermal steroids persist in a crystalline form for long periods of time (Atkinson, Suskind & Goldman, 1956). Unfortunately, this type of treatment resulted in substantial atrophy around the injected sites.

Topical application of steroid under occlusion also resulted in resolution of the lesions and removal of mast cells (Barton *et al.*, 1985). It is pertinent to note that in UP, mast cell depletion and reduction of histamine levels were achieved without continuous occlusion. This suggests that either diprolene is more potent than other steroids used previously, that mast cells in patients with UP are more susceptible to steroids, or that continuous occlusion is not necessary for mast cell depletion to occur.

The most surprising and potentially significant finding of this investigation revolved around the delayed return of mast cells after stopping treatment. Patients with UP had almost complete remission of the lesions. The treatment course was 6 weeks, and within 12 weeks of stopping treatment the lesions and hyperpigmentation disappeared. Clinical remissions have lasted an average of 11·5 months to date and they may continue longer with further observation. The high pre-treatment levels of mast cells observed in lesions were not detected in biopsies 6, 12, 18 and 24 weeks after therapy was stopped, whereas in the previously cited normal human skin, mast cells were observed within 14–21 days post-treatment. The long-lived remission in cutaneous mast cell disease resulting from aggressive topical steroid treatment has substantial clinical implications. This type of treatment can be thought of as 'pulse-therapy' to the skin, and can be repeated when symptoms reappear.

Another intriguing finding involves changes in lesional pigmentation after discontinuation of steroid treatment. Lesions of UP are characterized by a heavily pigmented epidermis and a dermis filled with mast cells. At the end of 6 weeks of topical treatment, no mast cells were observed in the lesions, and hyperpigmentation still remained. Absence of mast cells persisted at 6, 12, 18 and 24 weeks after therapy was discontinued. Twelve weeks after treatment, however, a maximal decrease in hyperpigmentation was noted which lasted for the duration of the study. Concomitantly, melanocytes continued to have an effete appearance, and basal keratinocytes did not contain as many melanosomes when compared with pre-treatment biopsy specimens. These pigmentary changes in post-steroid UP patients are different from those observed after steroid treatment of normal skin. It is well known that steroids inhibit melanin synthesis without being melanocidal (Kligman & Frosch, 1979; Nordlund, Ackles & Traynor, 1981). In our studies on normal skin, removal of the steroid resulted in rapid restitution of functional melanocytes, with the result that there was actually hyperpigmentation 14 days post-steroid. The reaction of melanocytes and repigmentation after steroid treatment in UP is intriguing and suggests perhaps a functional difference in the melanocytes of UP skin and normal skin. Alternatively, a melanocyte/mast cell relationship might exist that was previously not appreciated.

## CONCLUSIONS

The studies presented here establish that steroids have an effect on at least two of the cellular elements of the dermis: an inhibitory effect on the synthetic ability of fibroblasts which results in the depletion of ground substance and consequent reorganization of the fibrous elements, and a toxic effect on mast cells as evidenced by mast cell disappearance. This latter effect provides a reversible *in vivo* system to study correlations such as those described, as well as to further study the biology of the mast cell. Furthermore, aggressive treatment with topical steroids of mast cell-dependent disorders may have a role in appropriately severe cutaneous disease states.

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## DISCUSSION SUMMARY

The following points were made:

(1) Pharmacological attempts to evaluate the response in the absence of mast cells (corticosteroid treated skin) have not yet been made.

(2) Also in favour of the disappearance of mast cells is the fact that in the treatment of urticaria pigmentosa, the decrease in histamine content is even more dramatic, and in some patients, even after a year, neither rise in histamine content nor return of mast cells has been observed.

(3) In urticaria pigmentosa, using the same protocol (leg-leg or arm-arm), very good results have been obtained with topical application. Trials with whole body treatment are now being performed.

(4) Only the ground substance is lost, neither loss of collagen nor elastin has been detected during the course of the experiments. Collagen and elastin only collapse.

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# Skin toxicity and cellular metabolism: *in vitro* models

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Human cells in culture are being used with increasing frequency as *in vitro* models for studying the mechanisms of drug-induced toxicity. Changes in cellular properties such as the adhesiveness of anchorage-dependent cells to their culture support, cell membrane permeability, proliferation, respiration and overall metabolic activity, have been monitored under the influence of a given drug by measuring, for example, cell detachment, release of intracellular material (ATP, proteins, DNA), exclusion of trypan blue or uptake of vital dyes, inhibition of thymidine incorporation and protein biosynthesis, as well as alterations in gas exchange and heat production.

In order to evaluate the applicability of this approach to skin pharmacology and toxicology, we have studied the effects of treatment with three different classes of antipsoriatic drugs (anthralin, 8-methoxypsoralen in combination with UVA, retinoids) on cultured human dermal fibroblasts and/or transformed epidermal keratinocytes. These drugs are known to exert certain dermatotoxic effects such as irritation, pruritus or skin dryness.

The present review of these studies discusses the use of cell culture models for investigating the mode of action of dermatotoxic compounds, relates the antipsoriatic activity of anthralin and PUVA to their cytotoxic potential, and deals with the problem of the extent to which, and under which conditions, *in vitro* results can be extrapolated to the situation *in vivo*.

## ANTHRALIN

Although anthralin (cignolin, dithranol, 1,8-dihydroxy-9-anthrone) has been used for several decades in the treatment of psoriasis, its mode of action is still obscure. Essentially, its interaction with the following cellular targets has been considered responsible for the anti-hyperproliferative effect of the compound: (i) DNA replication (Swanbeck & Lidén, 1966) and repair synthesis (Clark & Hanawalt, 1982), (ii) mitochondrial respiration (Raab & Patermann, 1966), and (iii) key enzymes of metabolic pathways such as glucose-6-phosphate dehydrogenase in the pentose shunt (Hammar, 1970), or lipoxygenase in the arachidonic acid cascade (Bedford, Young & Wagner, 1983). Since interference with most of these targets will affect cell growth and proliferation and, at higher drug concentrations, cell viability, it is reasonable to assume that the primary target should be the most sensitive one. Furthermore, the potential targets should be affected at drug concentrations which are found in human skin *in vivo* after application of therapeutic doses, i.e. for anthralin in the micromolar range (Kammerau, Zesch & Schaefer, 1975). Evaluating from this viewpoint the data available in the literature, DNA replication and

TABLE 1. Inhibition of different fibroblast functions by anthralin

| Parameter | Studied by: | $ID_{50}$ ($\mu$M) |
|---|---|---|
| Protein content/culture | Spectrophotometry | 6 |
| Attachment | Microscopy | 6 |
| Heat production | Microcalorimetry | 7 |
| Thymidine incorporation | Liquid scintillation counting | 0·5 |
| Oxygen consumption | Manometry | 1 5 |

cellular respiration appear to be the most plausible candidates. However, since this data has been obtained using different biological systems under different conditions, we performed a comparative study using human dermal fibroblasts in culture (line 7000, Flow Laboratories, Irvine, Scotland).

Thymidine incorporation into the trichloro acetic acid insoluble pool was taken as a measure for DNA replication (Jacques & Reichert, 1981) and respiration was assessed via oxygen consumption of the cultures (Pätel, Schaarschmidt & Reichert, 1981). The cytotoxic potential of the drug was evaluated by determining its influence on cell detachment, protein content per culture and the heat production of the cells. For the latter a special microcalorimetric technique was developed (Schaarschmidt & Reichert, 1981).

Table 1 summarizes the $ID_{50}$ values obtained for the different endpoints, all of which reacted in a dose-dependent manner. The three markers of cytotoxicity gave practically the same $ID_{50}$ value of about 6 $\mu$M; respiration, however, was four-fold, and thymidine incorporation even 12-fold more sensitive. According to these findings, DNA replication may be considered to be the primary target of anthralin action. However, for technical reasons, we had to apply different conditions for the study of these different parameters. Owing to the insensitivity of the manometric technique, a high cell titre, i.e. confluent cultures, had to be used to determine oxygen consumption, whereas the measurement of thymidine incorporation required growing, non-confluent cells. Thus, a different sensitivity of the cells to the drug in question, during different stages of growth, could have resulted in misleading data.

In order to check for this possibility we used another cell type, transformed human keratinocytes (line SV-K14; a kind gift from Dr Birgit Lane, London), which express in culture a hyperproliferative character and possess an impaired capacity to differentiate (Taylor-Papadimitriou *et al.*, 1982; Bernard *et al.*, 1985). SV-K14 cells share with normal epidermal cells the metabolic peculiarity that glutamine constitutes one of the major respiratory energy sources (Reichert *et al.*, 1983), whereas glucose is mainly oxidized via the pentose shunt (Freinkel, 1960). Thus, the comparative measurement of $^{14}$C-carbon dioxide release from $^{14}$C-glutamine and $^{14}$C-glucose can serve to assess simultaneously cellular respiration and the functionality of the cytosolic pentose phosphate pathway. Adding $^{3}$H-thymidine incorporation as a marker for DNA synthesis and the protein content per culture as an endpoint for cytotoxic activity, all four parameters can be determined in the same experiment under identical conditions. A detailed description of the experimental design is given elsewhere (Reichert & Shroot, 1986).

The results of a series of such experiments are summarized in Table 2. In contrast to the results obtained using dermal fibroblasts, respiration appears to be more sensitive than thymidine incorporation to anthralin treatment. The activity of the pentose shunt is less affected, ruling out glucose-6-phosphate dehydrogenase as a primary target of anthralin action.

TABLE 2. Inhibition of different keratinocyte functions by anthralin

| Parameter | $ID_{50}$ ($\mu$M) |
|---|---|
| Protein content per culture | 8 |
| Thymidine incorporation | 6 |
| Carbon dioxide release from glucose | 6 |
| Carbon dioxide release from glutamine | 1 |

Since the results have been obtained under strictly identical conditions, we are now obliged to consider the antirespiratory effect of anthralin as the main reason for its antisporiatic action.

This hypothesis received further support from recent electron microscopic studies (Morlière *et al.*, 1985; Schubert & Christophers, 1985) revealing fast morphological changes at the mitochondrial level after application of anthralin formulations to human skin. These changes can be explained by our findings on the intracellular distribution of radiolabelled anthralin and its conversion products in cultured SV-K14 keratinocytes (Reichert *et al.*, 1985). The studies showed that a short incubation time of the cells with anthralin (approximately 15 min) is sufficient to trigger the full biological response, which manifests itself several hours later. During the first minutes of contact with 10-$^{14}$C-anthralin, the radioactive material could be almost completely recovered from plasma membranes and a particulate fraction consisting mainly of mitochondria and lysosomes. Essentially, no label was found in the cytosol. With increasing incubation times the radioactivity was increasingly accumulated within the particulate fraction (88% after 5 h). We were able to show that this accumulation occurs within the inner mitochondrial membrane system (unpublished results). Extraction of the cells after different incubation periods using organic solvents and separation of the extracts by radio-thin-layer-chromatography, and analysis of cellular proteins by SDS-PAGE, revealed that there is no stable adduct formation of proteins or lipids with anthralin, and that the radioactivity is predominantly recovered in the form of the polymeric oxidation product anthralin-brown. Some anthralin-dimer could be detected, the amount of which decreased with the incubation time.

Taking all these data into consideration, we postulate that the primary action of anthralin is mitochondriotropic and affects the cellular energy supply, which in turn slows down energy-dependent biosynthetic processes, including DNA replication. At higher doses of the drug, the depletion of metabolic energy becomes lethal and causes detachment of the cells from the culture support. Due to the rapid oxidation of anthralin and/or anthralin-dimer at the inner mitochondrial membrane, the compound becomes immobilized and is eliminated, *in vivo*, by desquamation. This would explain the low systemic toxicity of the topically-applied drug.

Extrapolating our *in vitro* results to the situation *in vivo*, we presume that optimal therapeutic effects will be obtained with doses that reduce respiration but yet are not lethal to the cells. Owing to the changing thickness of the epidermis, and due to the low ratio between the $ID_{50}$ values for the manifestation of cytotoxic effects and the inhibition of respiration (which we consider to be a useful *in vitro* guideline for the so-called 'risk-benefit ratio'), such an optimal situation is difficult, if not impossible, to maintain.

In order to improve this 'risk-benefit ratio' and to eliminate undesired side-effects such as staining of skin and clothes, the CIRD has undertaken a drug development programme based on

TABLE 3. Correlation between rabbit skin irritation and *in vitro* cytotoxicity induced by 41 anthranoids

| Irritation | Cytotoxicity | Fibroblast system | Keratinocyte system |
|---|---|---|---|
| + | + | 18 (44%) | 17 (41%) |
| + | − | 3 (7%) | 4 (10%) |
| − | + | 5 (12%) | 6 (15%) |
| − | − | 15 (37%) | 14 (34%) |

anthralin. This enabled us to relate the cytotoxicity of 41 anthralin analogues with their skin-irritating potential. Since many of these compounds were poorly soluble in aqueous media, 10 μM was applied routinely as the highest concentration in the *in vitro* test systems. The inhibition of thymidine incorporation was taken as an endpoint for cytotoxicity because it was, in general, more sensitive to treatment than the protein content of the cultures. A compound was classified as cytotoxic when its $ID_{50}$ value was 10 μM or lower. To assess their irritating potential, the analogues were applied on rabbit skin at a concentration of 0·1% in acetone. Compounds that provoked more than trace levels of irritation within the following 8 days were counted as positive (A.Chatelus, M.Dange & C.Hensby, unpublished observations).

The results of this qualitative study are summarized in Table 3. Although the criteria chosen are approximative, the correlation between *in vivo* and *in vitro* findings is surprisingly good. This holds true, however, only as long as the comparison is restricted to the chemically-related family of anthranoids. Other families of compounds, such as retinoids, irritate *in vivo* when the same protocol is applied, but are inactive in the *in vitro* systems.

## PUVA

The treatment of psoriasis by photochemotherapy with an orally-applied psoralen, and subsequent irradiation of the involved skin with long-wave ultraviolet light (Parrish *et al.*, 1974), is often chosen as an alternative to topically-administered anthralin. Most protocols today use 8-methoxypsoralen (8-MOP, methoxsalen) at oral doses of about 0·5 mg/kg body weight followed, 1–3 h later, by irradiation with 1–15 joules of UVA per $cm^2$, depending on skin type and pigmentation. The molecular basis of the PUVA treatment is thought to be the interaction of psoralen with cellular DNA. After irradiation, covalent monoadducts of cross-links with pyrimidine residues of the nucleic acid (Dall'Acqua *et al.*, 1971) are formed, which cause the inhibition of DNA replication and transcription and, finally, of cellular proliferation.

Although this concept is strongly supported by biochemical and electron microscopic data, other mechanisms remain under discussion in the literature, such as the photodynamic interaction of psoralens with membrane or mitochondrial proteins. Most recently, even a psoralen-specific receptor has been described for mammalian cells (Laskin *et al.*, 1985). It was therefore of special interest to investigate the response of our keratinocyte system towards the treatment with 8-MOP and UVA irradiation. Since this study has not yet been published in detail (Reichert, Shroot & Schaefer, in preparation), a description of the technical conditions is given in the following section.

Non-confluent cultures of SV-K14 cells were pre-incubated for 1 h with different concentrations of 8-MOP (up to 30 μM), and immediately thereafter exposed to UVA irradiation

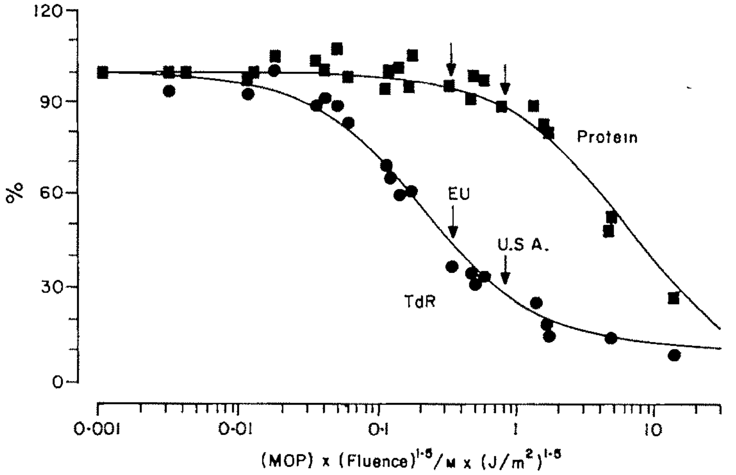

FIGURE 1. Thymidine incorporation and protein content per culture in the SV-K14 *in vitro* system relative to the product of 8-MOP concentration and (UVA fluence)$^{1·5}$. The arrows mark the positions of the average initial conditions applied in a European (EU) and an American (USA) co-operative clinical trial. ■ protein content of the culture; ● thymidine incorporation.

(Philips TL20W/09) at different doses ranging up to 0·6 J/cm$^2$. The biological response was assessed 24 h later. Under the above conditions, neither 8-MOP nor UVA alone gave rise to detectable biological effects. With 0·6 J/cm$^2$, the following ID$_{50}$ values for the reduction of thymidine incorporation, protein content per culture, glutamine respiration and glucose oxidation were obtained: 0·4, 11, 30, and ⩾30 $\mu$M, respectively. The high sensitivity of thymidine incorporation is in agreement with the assumption that the antiproliferative effect of PUVA is caused by the photodynamic interaction of the psoralen with cellular DNA.

A non-linear regression analysis of all the data obtained with varying 8-MOP concentrations and UVA doses further supported this view in revealing (Fig. 1) that the dose response of the most strongly affected endpoints, thymidine incorporation (TdR) and protein content per culture (PROT), can be described by the following equations:

$$\text{TdR}\,(\%) = 10 + \cfrac{90}{1 + \cfrac{(\mathit{8\text{-}MOP}) \times (\mathit{fluence})^{1·5}}{0·2}} \tag{1}$$

$$\text{PROT}\,(\%) = \cfrac{100}{1 + \cfrac{(\mathit{8\text{-}MOP}) \times (\mathit{fluence})^{1·5}}{6·55}} \tag{2}$$

with the 8-MOP concentration given in $\mu$mol/l and the UVA dose (*fluence*) in J/cm$^2$.

As can be seen from these equations, the biological system responds more strongly to variations of the UVA fluence, which is weighted with an exponent of 1·5, than to variations of the 8-MOP concentration, which is weighted with an exponent of 1·0. An exponent of 1 for the UVA fluence would be expected when the biological effects are caused by monoadduct formation, an exponent of 2 when only cross-links exert an action. The intermediate exponent of

1·5 indicates that both monoadducts and cross-links contribute to the biological response but with different efficacies (Grossweiner, 1981). Since the effect of thymidine incorporation is 30-fold more sensitive than the effect on protein content of the culture, and since both show the same kinetics with respect to UVA variations, we postulate that DNA is the primary target for the combined application of psoralens and UVA, and that the reduction in the protein content is the consequence of this, in principle, cytotoxic interaction.

How do the results of our *in vitro* study compare with the protocols for the clinical PUVA treatment of psoriasis? There are numerous reports concerning serum or plasma levels of 8-MOP after oral administration of therapeutic doses. These concentrations are in the order of 1 μM at the time of UVA irradiation (Christophers & Pohl, 1981), and are supposed not to differ markedly from the skin concentrations. Average initial UVA doses, prior to UVA-induced hyperpigmentation during the course of the treatment, range between 1·85 J/cm$^2$ (a European multicentre study involving 3050 patients; Henseler *et al.*, 1981) and 3·4 J/cm$^2$ (the corresponding U.S.A. study involving 1308 patients; Melski *et al.*, 1977). Assuming a sinoidal variation of epidermal thickness between 40 and 140 μm, and considering that the approximative depth of penetration of UVA irradiation in fair caucasian skin is 60 μM for 1/e (36·8%) of the incident energy density (Anderson & Parrish, 1982), one can calculate that, on average, about 25% of the applied UVA dose will reach the germinative epidermis. Inserting the appropriate values in Equations (1) and (2), we can state (Fig. 1) that, using the therapeutic *in vivo* conditions, the effect on the *in vitro* system is to strongly inhibit keratinocyte proliferation (as assessed by the inhibition of thymidine incorporation), but yet alter very little the cellular viability (as judged by the reduction in the protein content per culture). Thus, the therapeutic protocol, developed by experience, relates quite well to the optimum 'risk-benefit' conditions found in the *in vitro* system.

## RETINOIDS

Trans-retinoic acid was introduced into dermatology for the treatment of hyperkeratoses, first in topical formulations (Stuettgen, 1962), and later in topical and oral combinations (Schumacher & Stuettgen, 1971). Topical application caused skin irritation and oral administration led to severe systemic side-effects. The obvious therapeutic potential of the drug, however, led to the development of synthetic analogues, with the objective of maintaining or even increasing the therapeutic efficacy, but diminishing the adverse side-effects (Bollag & Geiger, 1985).

The retinoids are of special interest for the validation of our *in vitro* approach because they reveal its limitations. They exert strong cutaneous and systemic toxic effects under therapeutic conditions, but are, however, ineffective in the cell culture systems at concentrations up to 10 μM, which by far exceed therapeutic levels (Lauharanta & Paravicini, 1982). Therefore, in contrast to anthralin and PUVA, this group of antipsoriatic compounds most likely acts via interference with the complex mechanisms involved in the control of epithelial cell differentiation, and not via direct inhibition of the above-mentioned biochemical pathways.

There are, nevertheless, *in vitro* systems which are able to display the pharmacological activity of retinoic acid and its analogues. The potential of these compounds to modulate keratin synthesis and to inhibit the formation of a cornified envelope during the maturation of epidermal cells is of direct dermatological relevance. This property had led to the development of a specific bioassay for retinoids (Gilfix & Green, 1984), which we adapted to the transformed human keratinocyte line SV-K14. These cells, despite their limited capacity to differentiate, are

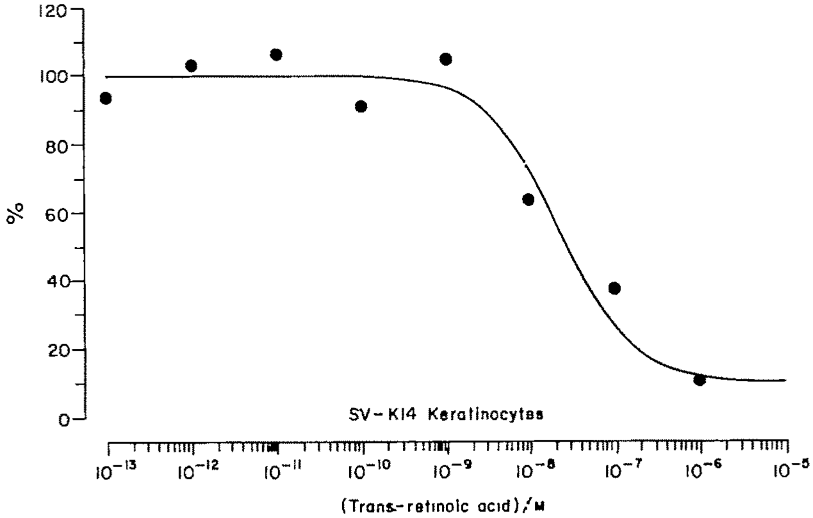

FIGURE 2. Dose response of the inhibition of calcium ionophore-induced cornified envelope formation by trans-retinoic acid.

able, under certain conditions, to synthesize a cornified envelope (Schmidt *et al.*, 1985), and this capability is suppressed in the presence of retinoids (Fig. 2). The $ID_{50}$ values determined with this system for a number of retinoids are in the submicromolar range, which correlates with therapeutic skin concentrations.

## CONCLUSIONS

The results obtained on the action of anthralin, PUVA and retinoic acid demonstrate that cell culture systems can be used: (i) to study pharmacological and cytotoxic mechanisms; (ii) to screen chemical modifications of a given drug, and (iii) to obtain data which can be extrapolated to the situation *in vivo*. However, the validity of these systems depends on: (i) the knowledge of the target cell *in vivo*; (ii) the condition that the drug is either not metabolized or undergoes the same conversion *in vitro* as *in vivo*, and (iii) the prerequisite that the drug exerts its activity *in vitro* at concentrations that are found *in vivo*.

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## DISCUSSION SUMMARY

The study was started with normal human keratinocytes but the data were not comparable from one experiment to the other due to the inherent variability of different skin sources. Nevertheless, the metabolic balance and the response to anthralin of the transformed SV-K 14 cell line was essentially the same as those observed with normal human keratinocytes.

It is not known whether cytochrome P 450 is present in the cells used in this study. However, the participation of cytochrome P 450 in anthralin action is unlikely since the cellular fraction from which most of anthralin and its conversion products are recovered does not contain the microsomal fraction where cytochrome P 450 is normally located.

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# Endpoints in short-term tests: significance for carcinogenic potential

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During the past decade, considerable effort has been directed towards the development and evaluation of short-term test systems for use in determining the carcinogenic potential of chemicals. The number of different systems proposed, and used to varying degrees, has been reported to be over 100 (Hollstein *et al.*, 1979). However, from among this array of test systems, only a relatively small number has had any significant or widespread use. Several factors have contributed to the process of test selection, and include the ease with which the assay can be performed, the data base providing information on the predictive value of the tests, and the nature of the endpoint measured. The endpoints can be measured in both prokaryotic and eukaryotic cells, and the effects of chemical interactions at the different cellular levels are expressed as gene mutation, DNA damage and repair, chromosomal damage and cell transformation.

Although several different endpoints, including mutation, DNA damage and chromosomal effects, can be used to assess the interaction between various micro-organisms and chemical carcinogens, the endpoint most frequently evaluated is mutation induction. The microbial mutations are detected as phenotypic changes and can result from alterations in the structure of DNA such as base substitutions, frameshifts, large deletions, insertions and translocations. The most extensively studied and widely used microbial gene mutation assay is the Salmonella/mammalian microsome test (Maron & Ames, 1983). In this test, specially constructed mutants of *Salmonella typhimurium*, blocked at various steps in histidine biosynthesis, are used to detect reverse mutations. In general, the test chemical and bacteria of the different tester strains, both with and without liver enzyme S9 for metabolic activation, are incorporated in soft agar and plated on a base of selective agar. The mutants arising after chemical treatment result mainly from basepair substitution or frameshift mutations, and grow in the absence of histidine. This assay is sensitive and versatile and can be used to evaluate many types of substances, including liquids, solids, gases and highly toxic substances.

Other micro-organisms which have been used for testing, but to a lesser degree, include various strains of the bacteria *Escherichia coli* (Mohn, Ellenberger & Van Bladeren, 1980), the yeast *Saccharomyces cerevisiae* (Zimmerman, 1984), and the mould *Neurospora crassa* (deSerres & Malling, 1983). A general problem encountered with both yeast and mould is the lack of sufficient permeability of the cell wall, which can restrict entry of certain chemicals into the cell. In addition, eukaryotic micro-organisms usually require higher chemical concentrations for induction of mutations than do bacteria, possibly because yeasts and moulds have more powerful detoxification mechanisms and more DNA repair pathways.

Gene mutation can also be conveniently measured in several mammalian cell systems. The cells most frequently used include L5178Y mouse lymphoma (Clive *et al.*, 1983), Chinese hamster ovary (CHO) (Hsie *et al.*, 1981), and Chinese hamster lung (V79) (Bradley *et al.*, 1981). In L5178Y cells, the marker generally used to detect the mutagenic event is resistance to BUdR or trifluorothymidine, which results from a loss of thymidine kinase activity. The marker most commonly used in CHO and V79 cells is resistance to 6-thioguanine or 8-azaguanine, which arises from a loss of hypoxanthine guanine phosphoribosyl transferase (HGPRT) activity. Resistance to ouabain, which results from a mutation affecting a membrane-associated sodium/potassium ATPase, can also be measured in V79 cells. Since all of these cell lines have limited capacity to metabolize chemicals to their ultimate reactive form, exogenous metabolic activation is provided, either by liver enzyme S9 preparations or intact cells; both primary fibroblasts (Huberman & Sachs, 1974) and liver epithelial cells (Langenbach, Freed & Huberman, 1978) can be used. Cell-mediated mutagenesis appears to be a better indicator of *in vivo* metabolic pathways (Selkirk, 1977; Schmeltz, Tosk & Williams, 1978; Dybing *et al.*, 1979; Bigger *et al.*, 1980) and may also reflect the organ specificity of the chemicals tested.

A variety of techniques can be used to measure DNA damage and consequent DNA repair. Strand breakage can be assessed either by alkaline sucrose gradient centrifugation (McGrath & Williams, 1966; Lett *et al.*, 1967) or by alkaline elution from membrane filters (Swenberg, Petzold & Harback, 1976; Kohn, 1979). However, care is required in interpreting the results of such assays, since dead or dying cells will display DNA fragmentation as a result of the action of degradative nucleases. Repair synthesis or unscheduled DNA synthesis can be measured either by autoradiography (Rasmussen & Painter, 1964; Painter & Cleaver, 1969) or by liquid scintillation counting (Evans & Norma, 1968; Stich & San, 1970). However, only with autoradiography is it possible to distinguish repair synthesis readily from replicative DNA synthesis, and this technique is preferred for screening (IARC, 1980).

Among the cell types that have been used for repair studies are continuous cell lines such as HeLa (Martin, McDermid & Garner, 1978) and WI-38 (San & Stich, 1975). However, major problems are associated with the use of cell lines. These include the need to inhibit replicative DNA synthesis and the need for an exogenous system for metabolic activation. Both of these problems have been overcome with the development of a DNA repair assay in primary rat hepatocyte cultures (Williams, 1976; 1977). The freshly isolated liver cells are non-dividing, have the capacity to metabolize carcinogens and respond with DNA repair. Studies to date have shown that this assay has substantial sensitivity in the detection of activation-dependent carcinogens (Probst, Hill & Bewsey, 1980; Williams, 1980; Williams, Laspia & Dunkel, 1982).

A number of different assays have been used for detecting chromosome damage, and the events evaluated include chromosomal aberrations, sister chromatid exchange, and micronucleus formation. Although a broad spectrum of cell types can be used for such evaluations, the cells most frequently used have been established lines of Chinese hamster cells, human peripheral blood lymphocytes and bone marrow cells of mice and rats. In general, Chinese hamster cell lines are particularly favoured because they have short cell cycles and a small number of easily distinguishable chromosomes.

Chromosomal aberrations (Preston *et al.*, 1981) induced in mammalian cells are of two types, namely, structural and numerical. The structural aberrations include both chromosome and chromatid types. A chromosome-type aberration is produced when the induced effect occurs in the G1 phase of the cell cycle when the chromosomes are single-stranded (single chromatid). A chromatid-type aberration is produced when the effect occurs in the G2 phase of the cell cycle when the chromosomes are double-stranded (two chromatids in each chromosome). Both chromatid and chromosomal types of aberrations involve deletions, duplications, rings and interchanges. Numerical aberrations include gain or loss of chromosomes (aneuploidy) and the occurrence of multiples of the diploid number or polyploidy.

Sister chromatid exchange (Latt *et al.*, 1981) involves symmetrical exchanges between sister chromatids of the same chromosome and does not result in gross alteration of chromosome morphology. The exchange events occurring between sister chromatids are visualized after the cells have been grown for two cycles in the presence of the thymidine analogue BUdR. Incorporation of the BUdR into DNA results in second division chromosomes having one chromatid unifilarly substituted and the other chromatid bifilarly substituted. After staining with a fluorescent dye and/or Giemsa, one chromatid (unifilarly substituted) appears dark, and the other (bifilarly substituted) appears light. The exchange events then appear as symmetrical light or dark staining areas in the dark or light staining chromatids (harlequin effect). The test can be performed on cells in culture, on cells from animals treated with the compound of interest or in the intact animal. In the latter case, a sufficient level of BUdR must be maintained to achieve the differential staining of the chromatids in cells of the tissue to be evaluated.

Chromosomal bodies that are not incorporated into daughter nuclei at the time of cell division are known as micronuclei (Heddle *et al.*, 1983). These are formed either from chromosomes as a result of the malfunction of the spindle apparatus or from chromosomal fragments. Polychromatic erythrocytes from the bone marrow are the cell type of choice. Erythroblasts, the precursor cells to erythrocytes, expel their nuclei after their last mitosis but retain their micronuclei. These micronuclei then appear as densely-staining, small bodies in the bluish staining polychromatic erythrocytes.

The last endpoint which can be used is morphological transformation. Cultured epithelial and fibroblastic cells can both be used for transformation, but the test systems most commonly used all employ fibroblasts. The morphological change, whether as altered colonies or foci in a monolayer of normal cells, is characterized by piling up of the cells in an irregular criss-crossed pattern, representing a loss of growth inhibition and cell-cell orientation. With passage, the transformed cells acquire other characteristics, such as the ability to grow in semi-solid medium, and the capacity to produce tumour after transplantation into syngeneic or immunosuppressed animals. The transformation system which probably has been most extensively used, as determined by the number of chemicals tested (Heidelberger *et al.*, 1983), is the Syrian hamster embryo clonal assay (Berwald & Sachs, 1963; DiPaolo, Nelson & Donovan, 1971; Pienta, Poiley & Lebherz, 1977). Primary or early passage cells are used as the target cell population. These cells are diploid and have enzyme systems which can metabolize a fairly wide spectrum of chemicals to their reactive forms.

Continuous cell lines, which can be used for transformation, include BALB/3T3 (DiPaolo, Takano & Popescu, 1972; Kakunaga, 1973; Sivak *et al.*, 1980) and C3H 10T1/2 (Reznikoff *et al.*, 1973). In assays with these cells, foci of morphologically transformed cells appear on a monolayer of normal cells. In contrast to hamster embryo cells, the mouse cell lines are hyperdiploid and have a more limited capacity to metabolize chemicals (Dunkel *et al.*, 1981; Heidelberger *et al.*, 1983).

Considering the endpoints available and the number of cell systems that can be used for measuring them, there is, naturally, concern about their biological relationship to *in vivo* carcinogenesis. Of all the endpoints, *in vitro* transformation can probably be considered as the most relevant because it corresponds most closely to the observed *in vivo* effect. Although the cells, when initially transformed, do not have the capacity to induce tumours after transplantation into the appropriate host, they do acquire this characteristic after a number of cell divisions. In certain respects, the *in vitro* progression of morphologically transformed 'preneoplastic' cells to a malignant state can be considered analogous to tumour progression *in vivo.*

The somatic mutation theory of cancer was the basis for the development and use of those tests with genetic endpoints. Some of the evidence to support this concept, and in turn, the use of genetic tests for determining carcinogenic potential, include the following:

(1) The conversion of a normal cell to a neoplastic cell results in a heritable change with the neoplastic characteristic transferred to daughter cells on division.

(2) Certain neoplastic diseases, such as neuroblastoma, retinoblastoma and Wilm's tumour, are inherited as autosomal dominant or autosomal recessive conditions (Mulvihill, 1975).

(3) Chromosomal alterations are observed in tumours, and such changes appear to occur non-randomly (Levan & Mitelman, 1977).

(4) The incidence of cancer is higher in individuals with certain genetic disorders such as Bloom's syndrome, Fanconi's anaemia and xeroderma pigmentosa (Mulvihill, 1975).

(5) Many carcinogens are metabolized to electrophilic species (Miller & Miller, 1966) which react with macromolecules including DNA, RNA and protein.

A number of aspects of carcinogenesis are inconsistent with the somatic mutation theory, however. Some agents, such as foreign bodies, produce tumours without evidence of direct interaction with DNA (Brand, 1982), and the incidence of tumours is higher in immunologically impaired individuals (Melief & Schwartz, 1982). In addition, experimental studies with mouse teratocarcinoma cells have shown that after these malignant cells are transplanted into mouse blastocysts, they give rise to a variety of normal tissues in mosaic mice (Mintz & Illmensee, 1975). The implication is that the neoplastic conversion did not involve a structural change in the DNA, but rather a change in gene expression. Since these other aspects of the carcinogenic process require consideration, justification for short-term tests cannot at present be based on mechanistic events.

In view of the correspondence between the *in vivo* effect and an *in vitro* endpoint as a reason for using cell transformation, and the somatic mutation theory of cancer as a reason for using genetic endpoints, one might be tempted to conclude that any single endpoint would be sufficient for making a decision about the potential carcinogenicity of a chemical. However, in the application of short-term tests, their significance resides, to a large degree, in the correlation between such tests and those obtained in well conducted and definitive long-term carcinogenicity bioassays. The purpose of correlation studies is to provide information necessary to gain a perspective for reasonable application of the methodology. The numbers from such correlation studies, however, cannot be isolated figures, but must be evaluated in the light of all relevant information.

In these comparative evaluations, the denominator of the correlation equation is derived from the results of *in vivo* carcinogenicity bioassays. The *in vivo* bioassay, as conducted by the U.S. National Cancer Institute/National Toxicology Program (NCI/NTP) protocol (Sontag, Page & Saffiotti, 1976), is a battery of four tests consisting of an experiment with the male mouse, female mouse, male rat and female rat. Although 50 animals are used per group, the bioassay is relatively insensitive and is designed to detect minimal levels of tumour incidences of 5–10% in the test group, as compared with none of the control (Chu, Cueto & Ward, 1981). In addition, there is well documented variability of response and assays about which no definite conclusion can be reached. Of 203 carcinogenicity bioassays [data summarized by Chu *et al.* (1981)] 67 gave no significant evidence for carcinogenicity and 15 were inadequate for evaluation. The other 121 assays fell into a number of different categories. In 20 assays (16·5%), there was positive evidence for carcinogenicity in both sexes of both species; in 23 assays (19·0%), there was positive evidence in at least one sex of both species; in 53 assays (43·8%), there was positive evidence in only one species, and finally, in 25 assays (20·6%), there was only suggestive evidence of a carcinogenic effect. This, then, is the standard against which *in vitro* tests are measured—a standard with its own set of difficulties and one which requires a mix of toxicological, pathological and statistical interpretation.

The numerator of the correlation equation is derived from the results obtained in short-term tests. An important factor here is the potential of any test to generate non-concordant results, which unfortunately have been referred to as false-negatives or false-positives. An extensive study conducted with one mutagenicity test, the Salmonella mammalian/microsome assay, provides some insight into the correlation that is obtained and other factors that must be considered when comparing *in vivo* and short-term test results (Dunkel *et al.*, 1985). In this study, a group of 60 chemicals, from among the more than 200 that had been tested by the NCI/NTP, were tested in this assay. All chemicals were from the same batch used for the *in vivo* bioassay, thus eliminating problems encountered with batch-to-batch variation. The *in vivo* results for this group of chemicals were as follows: 33 were carcinogens, 20 were non-carcinogens, and seven were designated as equivocal or suspect carcinogens. Twenty-five of the carcinogens (76%) were positive in the mutagenicity assay and 14 of the 20 non-carcinogens (70%) were negative. Among the group designated as equivocal or suspect carcinogens, five (71%) were positive and two (29%) were negative in this mutagenicity assay.

Based on these data, one might be tempted to conclude that the performance of this assay was adequate but not exceptional. Critical analysis of these data, however, underscores some of the factors that contribute to non-concordant results. For at least two of the carcinogenic chemicals, nitrilotriacetic acid trisodium salt (NTA) and reserpine, the lack of a mutagenic response may be partly related to the mechanism through which the chemical exerts its effects *in vivo*. In the *in vivo* bioassay of NTA, tumours of the urinary tract were observed at doses of 15 000 and 20 000 ppm. Such high doses have been reported to be associated with crystalluria (Anderson & Kanerva, 1978), and a causative relationship between crystalluria and urinary tract neoplasms was suggested. With reserpine, it is believed that hormonal mechanisms are an important consideration. It has been reported that long-term administration of this drug to women was associated with a greater than three-fold increase in the incidence of carcinoma of the breast (Boston Collaborative Drug Surveillance Program, 1974). It has also been found that reserpine increased the incidence of mouse mammary tumours (Lacassagne & Duplan, 1959) and promoted the growth of rat mammary tumours (Welsch & Meites, 1970). This effect has been linked to the ability of the drug to block the release of prolactin-inhibiting factor, causing a rise in serum prolactin level. The precise mechanism is not known, but if the action involves a hormonal mechanism, this compound would not be expected to act as a mutagen in bacteria. For that matter, any chemical causing cancer by a secondary mechanism would not be expected to be detected in microbial assays.

Another factor which affects the correlation is the presence of impurities in the test chemical. In the same study, 3-nitropropionic acid induced a strong mutagenic response but was designated as equivocal for carcinogenicity. The batch of chemical used in both the bioassay and the mutagenicity assay contained a 5% impurity which was identified as a dimeric ester of 3-hydroxypropionic acid (NCI-TR-52, 1978). This is a straight chain form of β-propiolactone from which 3-nitropropionic acid is synthesized. β-Propiolactone, which has been reported to be mutagenic (McCann *et al.* 1975), has high chemical reactivity and hydrolyses in water to 3-hydroxypropionic acid. The impurity was at a sufficient level to be detected in the mutagenicity assay, but may have been insufficient for adequate detection *in vivo*.

Correlations may also be influenced by the interpretation of the *in vivo* bioassay results. Two structurally-related aromatic diamines, 4-nitro-*o*-phenylenediamine and 2-nitro-*p*-phenylenediamine, were both mutagenic in *S. typhimurium*. The former chemical was judged not carcinogenic (NCI-TR-180, 1979) and the latter as carcinogenic (NCI-TR-169, 1979). Carcinogenicity was based on an increased incidence of hepatocellular neoplasms, primarily hepatocellular adenomas, in female mice. An increase in the same tumour type was also observed in female mice treated with the 'non-carcinogen', but the incidence was not statistically significant. Considering in this case the relative insensitivity of the *in vivo* bioassay and the fact that 4-nitro-*o*-phenylenediamine induces mutation in Salmonella as well as chromosomal aberrations and SCEs in mammalian cells (Shelby & Stasiewicz, 1984), one may well question whether the *in vivo* results are adequate for a final determination that this chemical is a non-carcinogen.

Responses that do not correlate with *in vivo* bioassays could also be related to the method used for biotransformation of particular substances to their ultimate reactive forms. Activating systems, either whole cells or the liver homogenates which are added to *in vitro* systems, cannot duplicate the entire metabolic capability of the intact animal, and it can be expected that some procarcinogens may fail to show activity in certain *in vitro* test systems. In other studies, it has been found that modification of procedures can provide the conditions necessary for generating the reactive compounds. For example, the carcinogenic glycoside cycasin is not a mutagen to Salmonella when tested in the presence of the liver S9 homogenate routinely used in the assay, but is mutagenic when $\beta$-glucosidase is added to the test system (Matsushima *et al.*, 1979). Similarly, mutagenic activity of diazo compounds derived from benzidine and its congeners *o*-toluidine, *o*-danisidine and 3,3'-dichlorobenzidine, is detected or enhanced when the assay system is modified to include the means for azo reduction (Prival & Mitchell, 1982; Prival *et al.*, 1984). In both of these examples, intestinal microflora are implicated in the activation of the parent compound to the reactive moieties.

Although a number of factors must be taken into consideration when using and interpreting the results from short-term tests, it is nevertheless apparent that such methods are of practical value in assessing chemicals for potential carcinogenicity. This area is still evolving and it can be expected, as we learn more about short-term test procedures, that there will be better definition of the methods and their application for control of chemical carcinogens in our environment.

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## DISCUSSION SUMMARY

In discussion Dr Dunkel made the following points:

(1) *In vitro* transformation assays do have the potential to be used in initiation/promotion studies. Dr Heidelberger has shown, for instance, that in 10T1/2 cells, TPA will promote 3-methyl-cholanthrene.

(2) For short-term studies, a battery of tests is used which generally includes a bacterial (Ames) test, but also assays for gene mutation in mammalian cells, unscheduled DNA synthesis, or chromosomal abberations.

(3) Equivocal test results are those in which the independent investigator felt unable to give a clear-cut yes or no answer, due to experimental variables, e.g. death of animals due to unexplained causes, or production of unusual tumours.

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# Three-dimensional culture of human keratinocytes on a dermal equivalent as a model system to study environmental modulation of epidermal physiology *in vitro*: effect of air-exposure

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For obvious reasons, the study of epidermal physiology is difficult *in vivo*. The use of conventional cultures of the major epidermal cell keratinocytes is not completely satisfactory because cells are attached to a non-biological sub-stratum (i.e. plastic) and are submerged in the culture medium. Moreover, under these conditions, their differentiation is not complete. More physiological conditions would be more meaningful for environmental and pharmacological studies. They would therefore require (i) the attachment of the cells to a biological matrix, and (ii) the contact of the tissue with the atmosphere. To achieve these goals, we have used the collagen-fibroblast dermal equivalent (Bell, Ivarsson & Merrill, 1979).

Human keratinocytes were first grown in primary culture under classical conditions and then seeded on dermal equivalents. In our study, we found that keratinocytes grown on such a dermal equivalent stratify and differentiate when raised at the liquid-air interface, while they remain as an undifferentiated monolayer when submerged in the culture medium (Fig. 1). To investigate this effect at the molecular level, we decided to study the modulation of the synthesis of the 67kD keratin polypeptide by the atmosphere. L-$^{35}$S-Methionine was added to (i) submerged cultures, (ii) air-exposed cultures, and (iii) air-exposed cultures submerged back in the medium prior to incorporation. Keratins were extracted and separated with SDS-PAGE, and the 67kD keratin was identified using the immunoblotting technique with KG8.13 monoclonal antibody (Gigi *et al.*, 1982). Autoradiograms of the immunoblots were also performed.

The results (Asselineau *et al.*, 1985) show that human keratinocytes grown on a dermal equivalent are able to express the 67kD keratin only if the culture is raised and maintained at the liquid-air interface (Fig. 1). Moreover, loss of contact with air results in switching off this synthesis.

In conclusion, we propose that this model is not only suitable for studying physiological environmental modulations, such as air exposure, but that it also represents a promising system for studying pharmacological modulations, particularly in the field of toxicological studies. Pharmacological agents can be added either on the air-exposed surface (equivalent to a topical application) or in the culture medium.

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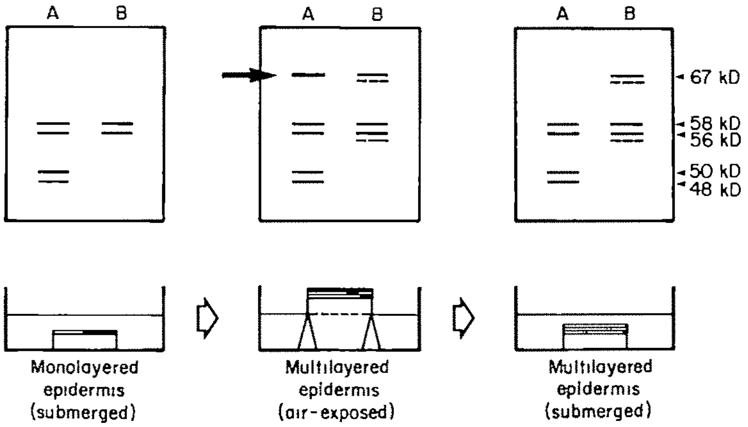
A
B
A
B
A
B
67 kD
58 kD
56 kD
50 kD
48 kD
Monolayered epidermis (submerged)
Multilayered epidermis (air-exposed)
Multilayered epidermis (submerged)

FIGURE 1. Schematic representation of the results. A: Autoradiography; B: blot.

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## DISCUSSION SUMMARY

The following points were made:

(1) The switch-off of the 67kD keratin synthesis occurs 'as soon as' the culture is put back into the medium, since methionine incorporation is performed at the same time.

(2) This switch-off is probably accompanied by that of the acidic 56·5kD keratin, which is the counterpart to the basic 67kD keratin. This is substantiated by a parallel decrease in both keratins and the appearance of degradation products.

(3) It is not yet clear whether the amniotic fluid contains any factor that might stimulate keratinocyte differentiation.

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# Onc-gene expression in hyperplasia induced by tape stripping or by topical application of TPA

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## SUMMARY

Guinea-pig ears were treated topically with 20 nmol of 12-O-tetradecanoyl phorbol-13-acetate, or deprived of their horny layer by nine successive strippings. At different times after the treatment, the animals were sacrificed, the epidermis removed from the ears, and the RNA from the epidermis purified and analysed by dot-blot hybridization in order to assess and determine the amount of RNA able to hybridize to each one of the 18 onc-gene DNA probes.

The following probes were used: v-src, v-fps, v-yes, v-ros, v-myc, c-myc, v-erb AB, v-myb, v-mos, v-Ha-ras, v-Ki-ras, v-abl, v-fos, v-fes, v-fms, c-sis, B-lym, v-raf. At 0 h, expression of B-lym and of myc and fos is seen. ErbAB mRNA is detected between 10 min and 4 h after stripping, as well as after TPA application. B-lym mRNA is detected for up to 36 h after stripping and for up to 8 h after TPA application. C-myc mRNa is detected for up to 36 h after tape stripping, but only for the first hour after TPA application. RNA complementary to the other onc probes was not detected, and synthesis of RNA complementary to an actin DNA probe was observed for 8 h after TPA application.

Hyperplasia can be induced in guinea-pig ear epidermis by topical application of TPA (12-O-tetradecanoyl phorbol-13-acetate) (Bourin *et al.*, 1982), as well as by mechanical stripping of the horny layer.

Because of the implication of onc genes in the control of cell development and proliferation (Heldin & Westermark, 1984), we have analysed the amount of the RNA hybridizing to onc gene DNA probes in guinea-pig ear epidermis at different times after topical application of TPA or after stripping.

## MATERIALS AND METHODS

At 0 h, 20 nmol of TPA in 100 μl of acetone were applied to the dorsal aspect of the ears of one set of guinea-pigs, while the ears of the animals from another set were stripped with nine blenderm tapes in order to eliminate the horny layer. At different time intervals, the animals were sacrificed, their ears cut off and frozen in liquid nitrogen, and dipped 1 min in 50 μg/ml of $ZnCl_2$ in water at 60°C. The epidermis was then separated from the dermis and ground in 6M urea, 3M LiCl. After precipitation of the nucleic acids, the pellet was recovered in 7·5M Guanidine HCl, 5mM DTT, added with an equal volume of 0·1M TrisCl pH 8, 4% Sarkosyl and layered on a high density CsCl cushion. The RNA sediments through the cushion and is found in the pellet, while DNA and proteins do not enter the cushion. This is a modification of published procedures for RNA purification (Chirgwin *et al.*, 1979; Schweizer & Goerttler, 1980), adapted for extracting RNA from keratinocytes.

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The pellet was recovered in 10 mM TrisCl, pH 7·5, 10 mM NaCl, 0·1 mM EDTA, and RNA samples (20 μg) were dotted on cellulose nitrate paper and hybridized in 50% formamide, 5 × SSC at 42°C to $^{32}$P labelled, heat-denatured onc gene probes. The probes consisted of cloned fragments of onc genes which were amplified in bacterial plasmids, electrophoretically separated from the vector after appropriate restriction digestion, and radiolabelled. The blots were washed in stringent conditions (0·1 × SSC, 0·05% SDS at 50°C), air dried and

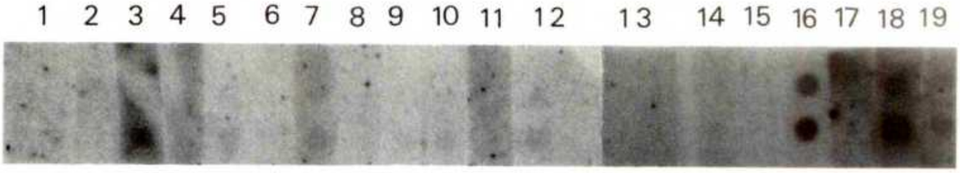

FIGURE 1. Dot-blot hybridization analysis of RNA from the epidermis of guinea-pig ears. Upper row: untreated control; lower row: 8 h after topical application of TPA. The probes used were: 1: v-src; 2: v-fps; 3: v-yes; 4: v-ros; 5: v-myc; 6: v-erb AB; 7: v-myb; 8: v-mos; 9: v-Ha-ras; 10: v-Ki-ras; 11: v-abl; 12: v-fos; 13: v-fes; 14: v-fms; 15: c-sis; 16: B-lym; 17: v-raf; 18: actin; 19: pBR322.

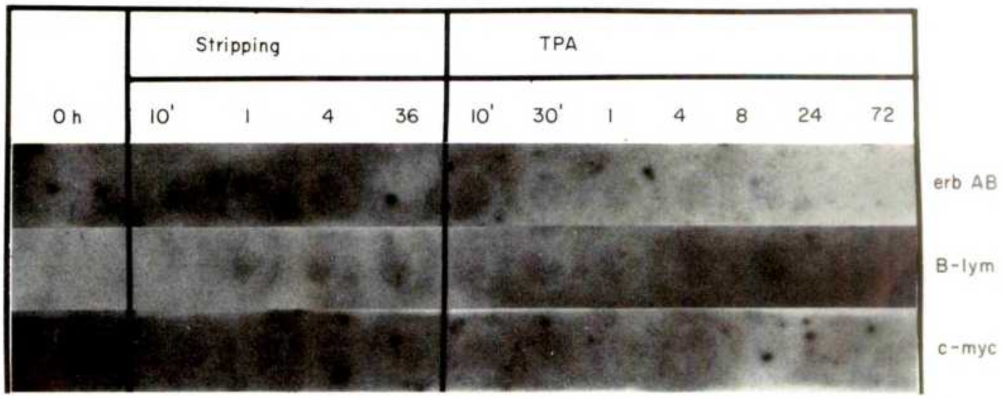

FIGURE 2. Dot-blot hybridization analysis of RNA from the epidermis of guinea-pig ears at different times after stripping or topical application of TPA.

autoradiographed (1 × SSC is: 0·15 M NaCl, 0·015 M trisodium citrate). For this dot blot hybridization analysis, the procedure described by Thomas (1980) was followed.

The onc gene DNA probes used in this study were: v-src, v-fps, v-yes, v-ros, v-myc, c-myc (human), v-erb AB, v-myb, v-mos, v-Ha-ras, v-Ki-ras, v-abl, v-fos, v-fes, v-fms, c-sis (human), B-lym (human) and v-raf. The actin probe consisted of mouse cytoplasmic cDNA for $\beta$-actin, cloned in pBR322 (a gift from Dr Alonzo, Institut Pasteur, Paris).

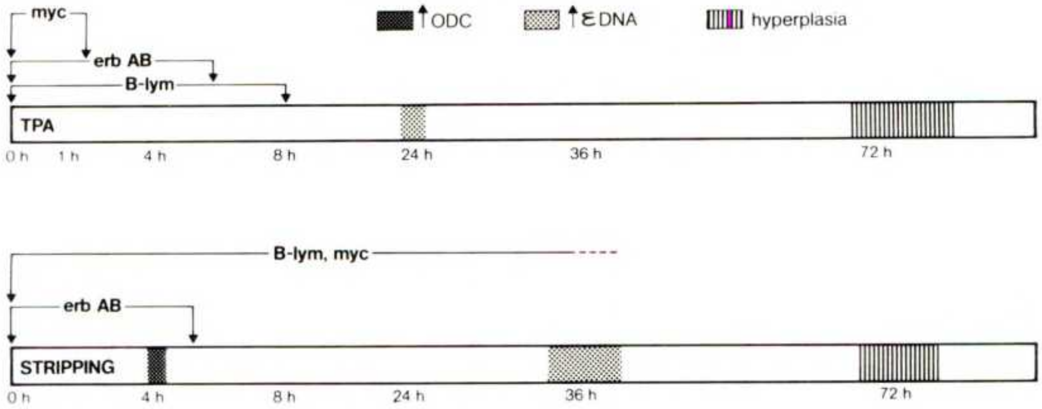

FIGURE 3. Comparison of the kinetics of induction of onc-gene expression to the kinetics of the appearance of biochemical phenomena in guinea-pig ears after stripping or TPA application. ↑ ODC indicates increase in the synthesis of ornithine decarboxylase. ↑ΣDNA indicates increase in the synthesis of DNA.

## RESULTS

The dot blot hybridization analysis of RNA from guinea-pig ear epidermis 8 h after topical application of TPA is shown in Figure 1. There is a striking increase in the relative content of RNA hybridizing to an actine probe, as well as to B-lym DNA. Closer inspection reveals that TPA induces an increase in the relative content of RNA hybridizing to v-myc and Ki-ras probes, while RNA hybridizing to v-myb and to v-fos appears to be very slightly expressed in the control and in TPA treated epidermis.

We then performed the analysis of the relative content of RNA hybridizing to the onc gene probes at different times after stripping or topical application of TPA. These results are shown in Figure 2.

It appears that RNA complementry to erb AB is expressed between 10 min and 4 h both after stripping and TPA application. RNA complementary to c-myc is expressed noticeably for up to 36 h after stripping, but only for the first hour after TPA application. Expression of RNA complementary to the other probes was not detected. RNA complementary to B-lym is expressed between 1 h and 36 h after stripping, and between 1 h and 8 h after TPA application.

The results are summarized in Figure 3 and compared to other known biochemical effects induced in the epidermis of guinea-pig ears by TPA (Bourin *et al.*, 1982) or by stripping (C.Delescluse, unpublished observation).

## DISCUSSION

While it might not be surprising to fail to detect the hybridization of onc gene probes to guinea-pig RNA, because of the sometimes low homology between viral and cellular onc genes and the highly stringent conditions used for the washing of the blots, it is interesting to observe the transient, though long-lasting, expression of RNA complementary to erb AB, since it is known that the sequence of erb B onc gene closely resembles the sequence of the gene coding for the receptor of the epidermal growth factor (Downward *et al.*, 1984).

Similarly, it is remarkable that RNA complementary to human c-myc onc gene is expressed for up to 36 h after stripping of the horny layer, and only for 1 h after topical application of TPA. C-myc onc gene is believed to play an important role in cell development and proliferation; for instance, it is known that it co-operates with Ha-ras onc gene in promoting the transformation of cells in culture (Land, Parada & Weinberg, 1983) and that it is hyper-expressed in the regenerating liver of rodents (Makino, Hayashi & Sugimura, 1984).

On the other hand, nothing is known of the role of B-lym, although one might wonder about its role in the epidermis, since it has been observed that it is expressed in normal human keratinocytes, but not in psoriatic keratinocytes (Giacomoni & Mansbridge, in preparation) and that its expression is stimulated by UV irradiation in normal human keratinocytes in culture (Giacomoni & Régnier, in preparation).

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# The hen's egg test (HET)—
# an alternative toxicity test

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The increasing variety and number of chemicals introduced onto the market and also into the environment each year, and the resulting requirements of protection of human health and human environment, have necessitated the monitoring of environmental materials and specimen banking, as well as the development of rapid and reliable methods for toxicity evaluation and risk assessment (Lüpke, 1979). Both ethical and legal obligations, e.g. animal protection laws, must be taken into account.

Chicken embryo models are well known and recommended as screening investigations in testing embryonic development and embryotoxicity; they show many advantages, but, nevertheless, their disadvantages must also be discussed. These basic evaluations have been extended and standardized in our laboratories in recent years by the development of the hen's egg test (HET; Hühner-Ei-Test) (Kemper, 1962; Lüpke, 1982). Hen's egg tests give information regarding embryolethality, embryonic and growth development, teratogenicity and systemic toxicity, including immunopathological aspects and metabolic pathways (Kemper, Lüpke & Renhof, 1982; Kemper & Lüpke, 1983).

This HET toxicity test has now been extended and standardized in the development of HET-chorionallantois-membrane test (HET-CAM test), as an alternative to the Draize *in vivo* rabbit eye test as a membrane irritation test (Lüpke, 1985). Regarding the actual discussion of reduction, refinement and replacement of animal experimentation, it is very important to stress that testing incubated hen's eggs is a borderline case between *in vivo* and *in vitro* systems, and does not conflict with ethical or legal aspects of animal protection.

## MATERIAL AND METHODS

Only fresh fertile White Leghorn eggs (Lohmann Selected Leghorn, LSL) are used in HET or HET-CAM tests; the eggs are incubated under optimal conditions. An overview of the frame of HET toxicity testing is given in Table 1; the method is described by Lüpke (1982). In the case of HET-CAM testing, on the 10th day of incubation, the egg shell is scratched around the air cell by a rotating dental sawblade, and removed. After careful removal of the inner egg membrane, the vital, vascular CAM, a membrane without sense of pain, is laid open; this is a modification of the technique described by Kemper (1958). The test substance, dissolved or suspended, is dropped on the CAM in a volume of 0·2–0·3 ml; in the case of solid test materials, 0·1 g is applied and irrigated after 20 s with 5 ml of warm water. After application, the CAM, the blood vessels, including the capillary system, and the albumin are examined and scored for irritant effects at 0·5, 2 and 5 min after treatment in a special apparatus for observation. The numerical time-

TABLE 1. Toxicity testing by HET

1. Lethality
   LD 50
2. Retardation
   (a) Hatching weight
   (b) Bone lengths (humerus, ulna, femur, tibia, metatarsus, skull thickness)
   (c) a–b correlation
   (d) Organ weights
3. Teratogenicity
   (a) Macroscopic-anatomical
   (b) Clearing and staining of skeleton
4. Systemic effects
   (a) Blood chemical parameters (Na, K, Ca, Cl, inorg. PO 4, proteins, creatinine, uric acid, glucose, cholesterol, triglycerides, GOT, GPT, LDH, AP, g-GT, bilirubin)
   (b) Haematological parameters (haemoglobin, haematocrit, blood cells, coagulation)
   (c) Organ weights and histopathology
5. Immunopathology
   (a) Thymus
   (b) Bursa of Fabricius
6. 1–5 investigations after application on day 1 (before incubation) and on day 5 (96 h after beginning)

dependent scores are summed to give a single numerical mean value indicating the irritation potency of the test substance on a scale with a maximum value of 21 (mean of a minimum of four tests); a rapid risk assessment is possible by a classification scheme analogous to the raw Draize categories (Table 2).

TABLE 2. HET-membrane irritation

Score for scaling

| | Time (min) | | |
|---|---|---|---|
| Effect | 0 5 | 2 | 5 |
| Injection | 5 | 3 | 1 |
| Haemorrhage | 7 | 5 | 3 |
| Coagulation | 9 | 7 | 5 |

Classification scheme

| Cumulative score | Assessment |
|---|---|
| –0·9 | Practically no irritation |
| 1–4·9 | Slight irritation |
| 5–8·9 | Moderate irritation |
| 9– | Strong irritation |

## RESULTS

To date, more than 100 substances have been tested under the aforementioned HET conditions. There is a very high correlation between findings in HET (Table 1) and results of mammalian investigations; this is true also for histopathological observations. In the special field of HET-CAM, approximately 190 substances and formulations have been examined in the aforementioned way in our laboratories. There is a good correlation between HET-CAM assessments and reported data based on Draize eye tests. Furthermore, there is a high reproducibility in HET-CAM tests between different investigators and laboratories at different times. Both Draize *in vivo* eye testing and HET-CAM tests are dependent on individual interpretations of tissue reactions; numerical scores may be sufficient for legal and administrative measurements on the one hand, but on the other hand, they are non-informative for toxicologists. Therefore, we recommend the use of a parallel yardstick of reference materials with well known irritation potencies, and, if possible, to give a supplementary descriptive characterization of the effects observed. In every case, the results of HET toxicity testing, as well as those of the HET-CAM testing, must be compared with untreated controls and normal values, as established in our laboratories over the last few years for different strains of White Leghorn eggs.

## DISCUSSION

HET is a rapid, sensitive and inexpensive toxicity test and provides information concerning embryotoxicity, teratogenicity, systemic and immunopathological effects, metabolic pathways and, in the form of the HET-CAM, membrane irritation potencies of chemical substances.

Testing incubated hen's eggs is a borderline case between *in vivo* and *in vitro* systems, and does not conflict with ethical or legal obligations, especially with respect to animal protection laws.

In the particular field of the HET-CAM testing, a special score and classification scheme was developed, which allows rapid risk assessments analogous to the raw Draize scheme based on *in vivo* rabbit eye tests. The results and assessments of both HET toxicity testing and HET-chorionallantois testing correlate well with *in vivo* findings in mammalian systems; this good correlation is also true for histopathological findings in HET and for interlaboratory double-blind studies in HET-CAM.

HET toxicity testing, as well as HET-CAM testing, cannot completely replace the currently-used toxicological tests on mammals in these fields, but they can diminish these investigations on mammals, as well as limit or eliminate pain and damage in animal experiments, and they allow one to set priority and toxicity categories and to identify target organs of toxicity.

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*British Journal of Dermatology* (1986) **115**, Supplement 31, 136–137.

# An evaluation of two approaches for dermatotoxicity testing

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Both an *in vitro* and an *in vivo* model are being evaluated for their potential use in skin toxicity testing. In the *in vitro* model, human epidermal cells were cultured to investigate effects on the cellular level. In the *in vivo* model, human skin was grafted onto athymic nude mice.

Multilayered cultures of human epidermal cells may serve as a model for the living part of the epidermis to test the effects of toxicants after penetrating the stratum corneum. To study the suitability of this culture system in assessing irritancy, membrane damage was measured after application of chemicals with different irritating potentials at a $10^{-4}$M concentration in Ham's F12. Severity of membrane lesions has been estimated by the determination of leakage of intracellular markers with a small ($K^+$) and a large [lactate dehydrogenase, N-acetyl-$\beta$-glucosaminidase ($\beta$NAG)] molecular size. After 4–24 h of exposure, a considerable loss of enzyme activity can be seen only with chemicals which act directly on the membranes, such as tributyltin and sodium dodecyl sulphate (SDS). The high sensitivity of lysosomal membranes to SDS (Imokawa & Mishima, 1979) is clearly expressed in a large decrease of intracellular $\beta$NAG. The intracellular level of $K^+$ appears to be a more sensitive indicator for cytotoxicity. Whereas after 0·25–2 h of exposure little enzyme leakage can be detected, considerable changes may occur in the $K^+$ content of the cultures. This is in agreement with results of Malik, Schwarz and Wiebel (1983), who used Chinese hamster V79 cells. A ranking order of the tested agents based on the results of these *in vitro* assays is in agreement with their known irritant effects on human skin.

The human skin grafted onto the nude mouse maintains its characteristic properties, as was shown by histological and immunofluorescent studies, and by the induction of microblisters by a thermal stimulus or with sulphur mustard. Percutaneous absorption was studied with the irreversible cholinesterase inhibitor soman, by measuring inhibition of blood cholinesterase (CHE) activity. Epicutaneous administration on intact and on auto- or homo-grafted nude mice showed that the penetration speed was not influenced by the grafting process itself. Penetration of 20, 60 and 100 μg soman/kg causes a dose-dependent inhibition of blood cholinesterase. At a suitable dose-level (60 μg/kg), the human skin appeared less permeable to soman than mouse skin. By comparing CHE activity after percutaneous absorption of soman with the activity after subcutaneous and intravenous injection, it appears that substantial amounts of the inhibitor are metabolized in the skin and subcutaneous tissues before reaching the bloodstream.

The results indicate that both the *in vitro* and the *in vivo* model can be used to assay dermatotoxic damage and to study mechanisms of action of toxic compounds.

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# Effects of an amphoteric surfactant on the skin barrier: an *in vivo* electro-physiological study

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In recent years there has been an increase in the commercial use of amphoteric detergents. As anionic/protein interactions are considered the basis on which irritation can occur, the addition of amphoteric surfactants has been proposed as a way of reducing the irritative potential of anionic surfactants (Garrett, 1965; Faucher & Goddard, 1978). Considerable inhibition of absorption of anionic surfactants on the stratum corneum has been demonstrated by amphoterics (Garcia *et al.*, 1981).

In this work a new approach is suggested to assess the effects of a commercial amphoteric detergent on the electrical skin barrier *in vivo*. The attempt was to investigate the ability of amphoterics to affect the skin's electrical barrier function and also to establish whether amphoterics represent a possible permeability factor for other agents.

## MATERIALS AND METHODS

Three compounds were present in the amphoteric used in this research (Candinet-Uniderm): alkylamidobetaine (Tego-Betaine L7), straw-yellow in colour and with a pH factor of between 3 and 5, and Tego 103G, which is made up of two compounds: an alkyldiethylene diaminoglycine and a dialkyldiaminoethylglycine. While L7 possesses both an emulsifying and detergent ability, the two Tego 103G components have a strong surfactant and an outstanding microbicidal action. The three compounds were each present in concentrations of 10%. Lactic acid, used to adjust the pH to 5·5, was also present in the product.

Ten female subjects aged between 17 and 24 years took part in the study. Concentrations of 10% and 5% were tested on the left forearm, and concentrations of 2·5% and 1·25% on the right forearm. The research was carried out using a new apparatus (Fig. 1) which made it possible to obtain the skin's locus of impedance (Bernardi, 1982). The apparatus generates sinusoidal frequencies of 100, 200 and 6000 Hz, which are sent to the skin through the electrodes. The patient receives a constant current and the signal we obtain in the output is proportional to the unknown impedance value. The phase angle and the impedance modulus are obtained by comparing them with a reference signal. By means of mathematical interpolation we can determine the size of an arc of a circle relating to the locus of impedance on a complex plane (Fig.

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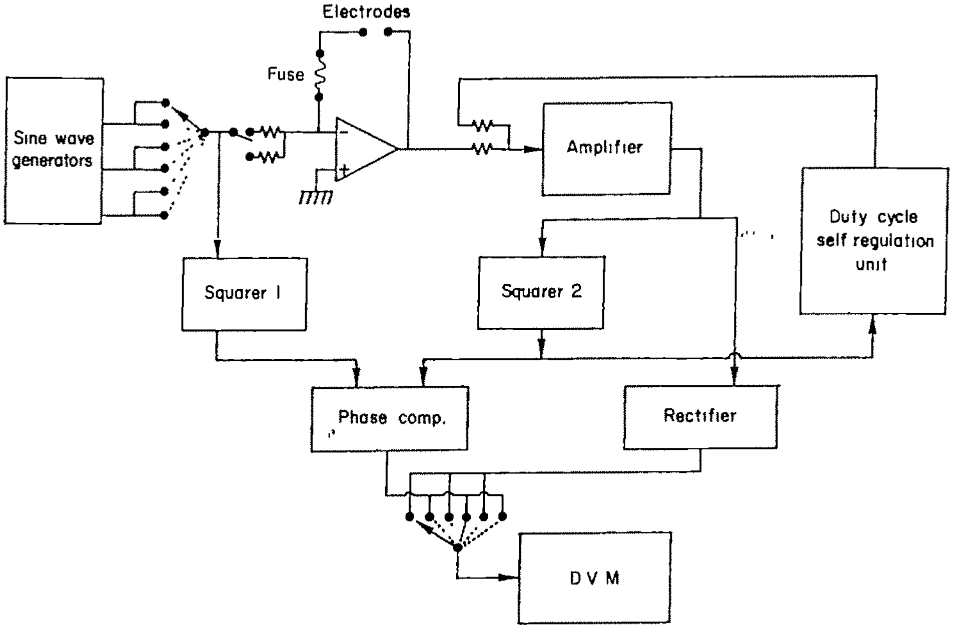

FIGURE 1. Block diagram of impedance device.

2). Ro (external resistance) can be determined by this geometric construction. This parameter is an expression of the stratum corneum's water content (Borroni *et al.*, 1982) and angle $\alpha$, whose limited size is typical of psoriasis, which is characterized by high permeability (Borroni *et al.*, 1983). Ro levels were measured in Ohms and angle $\alpha$ in degrees.

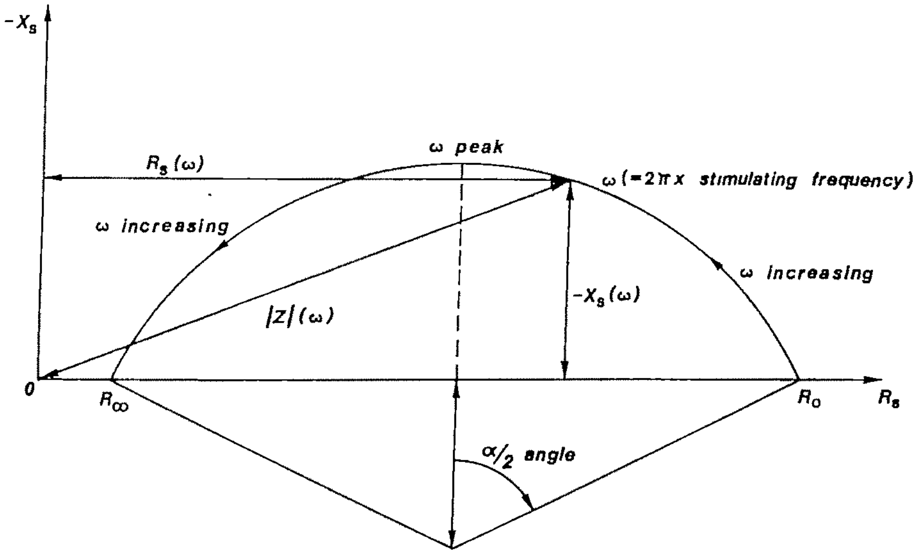

FIGURE 2. Skin impedance locus.

TABLE 1. External resistance (Ro) after applications of 5% and 10% amphoteric mixtures

| | Ro (OHMS)* | | |
|---|---|---|---|
| | | 10% | 5% |
| Left | 689 ± 579 | 297 ± 221<br>(NS) | 258 ± 275<br>(NS) |
| | | 2·5% | 1·25% |
| Right | 894 ± 399 | 250 ± 274<br>($P < 0{\cdot}01$) | 319 ± 207<br>($P < 0{\cdot}01$) |

* Mean values × 1000.

## RESULTS

The results are given in Tables 1 and 2. Table 1 demonstrates that at concentrations of 10% and 5% no significant variation in Ro was found. For the other concentrations (2·5% and 1·25%), a significant reduction ($P < 0{\cdot}01$) was noted in all the subjects tested. Table 2 shows that there was a decrease in the angle $\alpha$ in seven out of 10 subjects with respect to the basal data at 10% and 5% concentrations. The research yielded statistical evidence of $P < 0{\cdot}01$ for 10% concentrations and $P < 0{\cdot}05$ for 5% concentrations. With 2·5% and 1·25% concentrations there was a decrease in all cases with respect to the basal data. With both these concentrations the variations were statistically significant ($P < 0{\cdot}05$).

TABLE 2. Locus of impedance (angle) after applications of 5% and 10% amphoteric mixtures

| | Angle (degrees)* | | |
|---|---|---|---|
| | | 10% | 5% |
| Left | 146 ± 7 | 130 ± 11<br>($P < 0{\cdot}01$) | 124 ± 24<br>($P < 0{\cdot}05$) |
| | | 2·5% | 1·25% |
| Right | 144 ± 6 | 124 ± 20<br>($P < 0{\cdot}05$) | 130 ± 14<br>($P < 0{\cdot}05$) |

* Mean values.

## CONCLUSIONS

Variations in the electrical skin barrier induced by different concentrations of the amphoteric compound provided evidence of the following:

(i) the bathing effect of this amphoteric compound becomes more pronounced the more diluted the solution is. Significant Ro differences were recorded after the application of lower concentrations ($P < 0{\cdot}01$). Higher concentrations failed to produce significant variations.

(ii) Angle $\alpha$ shows statistical differences at every concentration with a decrease in the size of the angle ($P < 0{\cdot}01$ at 10% and $P < 0{\cdot}05$ at 5%, 2·5% and 1·25%).

Statistical differences were more marked at the highest concentrations. A significant reduction in the size of the angle was thus shown to be distinctive of a cutaneous condition (psoriasis), which is characterized by high permeability. The permeability of the skin to some substances can be assessed by varying the electrical parameters of skin impedance, in particular, the angle $\alpha$. The amphoteric detergent tested in this work modifies the stratum corneum's electrical state. The impaired functioning of the skin's electrical barrier would appear to be induced as a consequence of the increased presence of electrical charges under the stratum corneum.

(iii) Despite their high tolerability, when applied to the skin without careful washing, amphoteric detergents are potentially able to cause changes in the electrical barrier. The increase in electrical permeability may be regarded as a factor which increases the likelihood that topical agents, applied after these detergents have been used, will be able to penetrate the skin's barrier more readily.

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# Recent findings limiting immunological and photosensitizing hazards of the tetrachlorodecaoxygen anion complex (TCDO) in wound healing promotion

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The tetrachlorodecaoxygen anion complex (TCDO, $(Cl_4O_{10})_n^{-m}$) contains oxygen in a chemically soluble chlorite ($ClO_2^-$) matrix and improves oxygen supply to non-healing wounds (Hinz, Kühne & Stahl, 1984). TCDO has been shown to accelerate wound healing in a number of clinical trials (Hinz *et al.*, 1984; Hinz *et al.*, 1985; Hinz, Hautzinger & Stahl, 1986). This pharmacological property is apparently mediated (Hinz *et al.*, 1986) by an amplification (Ullman & Kühne, 1984) of physiological responses of the phagocytes which are actually considered to be key effector cells (Diegelmann, Cohen & Kaplan, 1981) in wound repair.

Topical applications of TCDO to chronic venous ulcers have resulted in a threefold acceleration of wound healing, i.e. wound surface decrease, in comparison to physiological saline. However, patients with such lesions constitute a high risk group for contact—as well as photoallergy. The purpose of this series of experiments was to assess: (i) the eventual phototoxic and photoallergic potential of TCDO with a predictive animal test, and (ii) the possible contact sensitization by TCDO in humans after epicutaneous administration.

## MATERIALS AND METHODS

### *Phototoxicity and photoallergy test*

Experiments were of placebo- and positive-test compound-controlled randomized design and all measurements were made under blind conditions. The model used was based on that described by Vinson and Borselli (1966). The tests were carried out on 25 approximately 8-week-old male Pirbright white guinea-pigs, plus two additional specimens to determine the highest non-irritating concentration of TCDO. Both the test compound TCDO (50%; 25% Oxoferin®*) and the substance 3, 3′, 4′, 5-tetrachlorosalicyl anilide (5% for phase I, 1% for phases II and III) were taken up in 0·1 ml of either ethanol (phase I) or peanut oil (phase II), or 2·0 ml of 8% soap solution (phase III), and applied either directly to a 7·5 × 5·0 cm² depilated

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* Trade name for the topical form of TCDO registered in the F.R.G. to support wound healing commercialized by OXO-Chemie, Heidelberg. One microlitre of this TCDO water solution contains 240 millimolar glycerine and the amount of TCDO which in the presence of $10^{-5}$M hemine fractionates 1·38 nanomoles ethylene from 1 aminocyclopropane carboxylic acid (ACC).

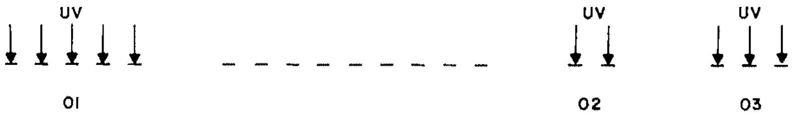
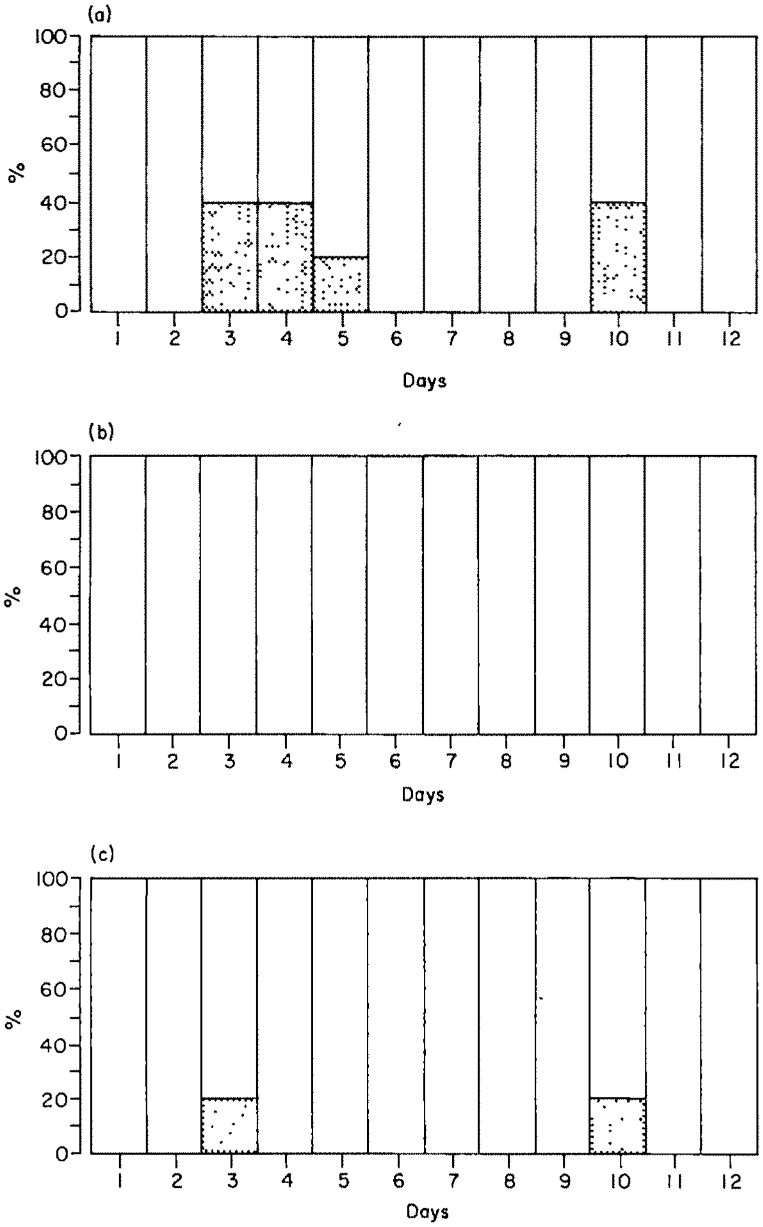


FIGURE 1. Treatment protocol for the phototoxicity/photoallergy test.



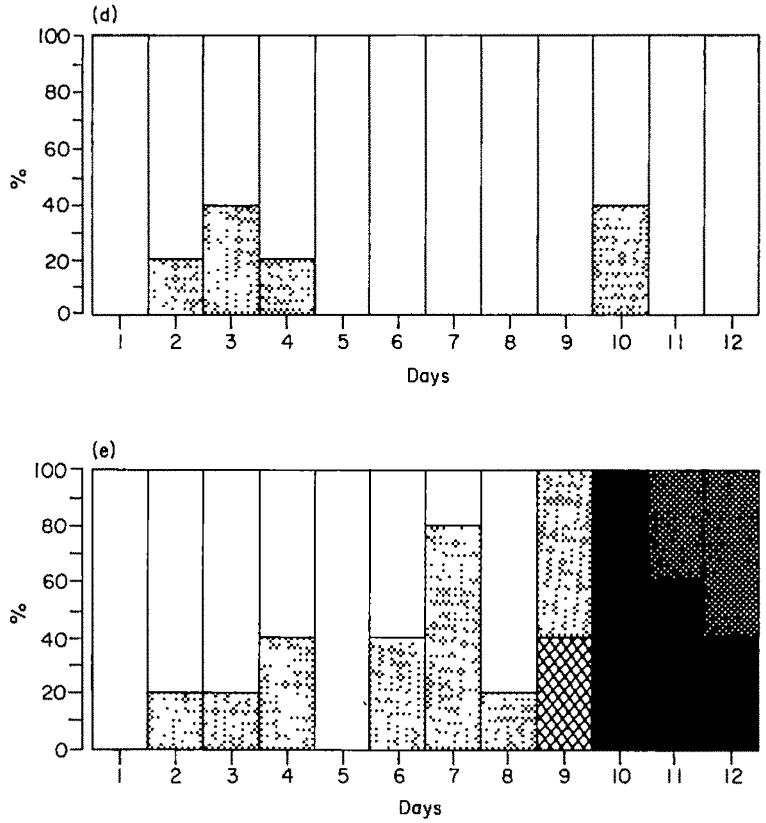


FIGURE 2. Phototoxic and photosensible reactions in the five guinea-pig groups specified in 'Methods', graded in five categories: 0 = none, 1 = borderline, 2 = slight, 3 = moderate, 4 = severe reaction. (a) Group 1: Oxoferin® 50% plus UV irradiation; (b) Group 2: Oxoferin® 50% without UV irradiation; (c) Group 3: Oxoferin® 25% plus UV irradiation; (d) Group 4: placebo control (vehicle plus UV irradiation); (e) Group 5: positive control tetrachlorosalicylanilide plus UV irradiation. □ = 0; ▨ = 1; ▦ = 2; ■ = 3; ■ = 4.

neck area (phases I, II), or indirectly to an equally depilated surface on the left flanks through a moistened patch (phase III) (right flanks as controls). The placebo group received only the solvents. During phase III, all animals received 2·0 ml of the soap solution on the right flanks. Except for Group 2, all animals were UV irradiated (Hanau Quarzlampe type Q 600) for 15 min immediately after exposure for 1 h to the test solutions. The groups were defined as follows: Group 1, 50% Oxoferin® + UV; Group 2, 50% Oxoferin®; Group 3, 25% Oxoferin® + UV; Group 4, ethanol + UV; Group 5, tetrachlorosalicyl anilide + UV. The treatment protocol was carried out according to Figure 1 leaving a 9-day rest period for eventual antibody formation and for skin normalization during phase II. Skin reactions were read 24 h after each application, and checked additionally 48 and 72 h after final treatment at the end of phase III.

*Epicutaneous tests*

Epicutaneous tests with Oxoferin® or the TCDO-free solvent of Oxoferin® were carried out on normal intact skin. Erythema reactions were graded in five categories: 0 = none, 1 = borderline,

TABLE 1. Results from patch tests in humans in the non-wound and wound patients. In the latter group, tolerance in the wound was also registered

| Patient Number | Sex | Age (years) | Oxoferin® | Oxoferin® solvent | Oxoferin® (erythema of wound border) | Contact allergic anamnesis |
|---|---|---|---|---|---|---|
| *Non-wound patients* | | | | | | |
| 1 | F | 36 | – | – | | |
| 2 | F | 77 | – | – | | |
| 3 | F | 28 | – | + | | |
| 4 | M | 27 | – | – | | |
| 5 | M | 57 | – | – | | |
| 6 | F | 49 | – | – | | |
| 7 | F | 24 | – | – | | |
| 8 | F | 28 | – | – | | |
| 9 | F | 45 | – | – | | |
| 10 | M | 50 | – | – | | |
| 11 | F | 32 | – | – | | |
| 12 | F | 18 | – | – | | |
| 13 | M | 27 | – | – | | |
| 14 | M | 27 | – | – | | |
| 15 | F | 26 | – | – | | |
| 16 | F | 15 | – | – | | |
| 17 | F | 23 | – | – | | |
| 18 | F | 26 | – | – | | |
| 19 | F | 67 | – | – | | |
| *Wound patients* | | | | | | |
| 1 | F | 19 | – | – | – | No |
| 2 | M | 87 | – | – | – | Yes |
| 3 | M | 70 | – | – | – | Yes |
| 4 | M | 62 | – | – | – | Yes |
| 5 | M | 51 | – | – | + | Yes |
| 6 | M | 75 | – | – | – | No |
| 7 | M | 57 | – | – | – | No |
| 8 | F | 90 | – | – | – | Yes |
| 9 | F | 73 | – | – | – | No |
| 10 | F | 76 | – | – | – | Yes |
| 11 | F | 68 | – | – | – | Yes |
| 12 | F | 84 | – | – | – | No |

2 = slight, 3 = moderate, 4 = severe reaction and trauma for testing contact sensitization in humans. Twelve patients with chronic venous ulcers (seven female and five male, mean age 68 years) and 19 non-wound patients consulting a university department of dermatology for diagnostic allergen testing (14 female and five male, mean age 36 years) were included. 5 ml of Oxoferin® or TCDO-free Oxoferin® solvent were poured on a 100 cm$^2$ piece of cotton gauze and the control and test compound patches were applied in a double-blind manner to both forearms where they were covered with a plastic foil and fixed with a rubberized bandage for 24 h. Reading of the skin reactions was carried out after 24, 48 and 72 h and graded as defined above.

For statistical evaluation of the ordinal data, the non-parametric Mann-Whitney test was applied.

## RESULTS

### *Phototoxic and photosensitizing potential*

Whereas the positive controls showed distinct erythema at the end of phase III, borderline reactions were registered in the UV-irridated animals having received local skin treatments with 25% and 50% Oxoferin®. However, in contrast to the positive controls, there was no sign of skin reactivity at the end of phase III and the borderline reactions during phase I did not differ significantly from the results registered in Group 4 (placebo controls having received ethanol + UV irradiation). Oxoferin® treatment alone without UV irradiation did not provoke any skin reaction at all.

### *Contact sensitization in humans*

As can be seen from Table 1, epicutaneous application of Oxoferin® did not provoke any skin reactions in humans, neither in non-wound patients nor in patients with chronic ulcer lesions. In one out of 12 patients of this latter group with a known contact allergic anamnesis, borderline erythema was registered at the site of the lesion, although the patient showed a negative result in the forearm patch tests.

## DISCUSSION

For moral and technical reasons, these experiments were conducted on a limited number of animals and human volunteers and, therefore, as such might be looked upon as being of a preliminary nature. However, the fact that more than 10 000 wound cases from surgeries and hospitals in West Germany have been monitored without any proven reports on phototoxic or contact allergic effects of TCDO wound treatment, emphasizes the following noteworthy points from these experiments:

(i) Oxoferin® diluted twice and four times showed neither phototoxic nor photosensitizing properties in guinea-pigs.

(ii) Undiluted Oxoferin® did not provoke any erythema reactions after epicutaneous patch application on the intact skin of human volunteers and wound patients. These findings are supported by biochemical *in vitro* experiments (Youngman *et al.*, 1985), showing that in the presence of hemoproteins, TCDO has short half-lives ($t_{50} = 30$–$90$ min) and decomposes entirely to the physiological metabolites, $O_2$, $Cl^-$, $H_2O$. They minimize the probability of any risk that, *in vivo*, TCDO might act as an immunogenic compound.

(iii) An essential pharmacological feature of TCDO in wound healing is the amplification of physiological metabolic responses in phagocytes (Hinz *et al.*, 1986). This, together with the recent results showing that TCDO increases the delayed hypersensitivity response in mice to sheep red blood cells (G.Gillissen, personal communication) bestows effective immunoadjuvant properties to this compound. The borderline erythema reaction in the wound of one patient with known contact allergenic anamnesis and negative patch reaction on intact normal skin cannot be ascribed to a direct allergenic potential of this compound, but it could be due to an enhancement of an immunogenic reaction against a co-existing allergen. This is the reason why Oxoferin® treatment of chronic wounds, especially leg ulcers, should always be conducted as a monotherapy.

## ACKNOWLEDGMENT

E.M.W. thanks OXOCHEMIE Gmbtt for subsidizing the clinical studies.

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# Index

Allergic contact dermatitis, 53, 82, 93
Allergic reactions, 24
Anaphylaxis, 24
Angry back syndrome, 93
Antiandrogenicity assay, 33
Antiandrogens, 36
Antiseptics, 41, 63

Benzoyl peroxide, 49
Butylated hydroxytoluene, 49

Calcium, 17
Carcinogenesis, 1, 9, 117
Cell cultures, 17, 53, 108, 126, 136
Chlorhexidine, 41
Clotrimazole, 9
Coal tar, 80, 86
Corticosteroids, 71, 101
Cytochrome $P_{450}$, 9

Dermis, 101
Dithranol, 80, 108
DNCB, 47, 53, 93
Draize test, 24
Drug metabolism, 9
Dyes, 41

Electrophysiology, 138
Enzyme inhibitors, 9
Epidermis, 1, 9, 17, 47, 63
Erythema, 86

Fibroblasts, 17, 108

Glutathione, 86

Hen's egg test, 133
Hydrocortisone, 71
Hypersensitivity, 24, 93

Interferon, 76
Irritation, 45, 80, 108, 136

Keratin, 126
Keratinocyte culture, 17, 108, 126

Lactate formation, 45
Leg ulcers, 142
Lindane, 63
Lymphocytes, 24, 53

Malathion, 49
Mast cell depletion, 101
Mechlorethamine, 82
Melanocyte culture, 17
Mitogenic test, 24
Models *in vitro*, 108
Mutagenicity, 63
Mutagenicity—bacterial, 86, 117

Nucleic acids, 1, 9, 108, 117, 128

Oxoferin, 142

Patch test, 24, 93
Percutaneous absorption, 36, 47, 49, 63, 71
Phorbol Esters, 1, 128
Phototoxicity, 86
Psoriasis, 82
PUVA, 82, 86, 108

Quail uropygial gland, 33

Retinoids, 108
RU 38882, 33

Salicylic acid, 63
Sebaceous glands, 33, 36
Stratum corneum, 63, 71
Surfactants, 138

Testosterone, 33
Thymidine incorporation, 86, 108
Topical treatment—security factor, 71

Ultraviolet light, 80

Warts, 76
Wound healing, 41, 142

# Advance Notice

The Seventh CIRD Symposium 'Advances in Skin Pharmacology' will be held at the Hotel Westminster, Nice, from Friday, 26 September to Sunday, 28 September 1986. The Symposium will focus on 'Skin Pharmacokinetics' in both animals and man for topically-applied drugs. Drug kinetics in the skin after systemic application will also be considered.

Emphasis will be placed on: the nature of the skin barrier, *in vivo/in vitro* correlations, and models and their standardization.

## PARTIAL LIST OF SPEAKERS

*Session I (Friday Afternoon): Structure and Function of Permeability Barriers*
Elias, P. (San Francisco)—Darmon, Y.M. (CIRD).

*Session II (Friday Afternoon): General Principles of Skin Permeability*
Stüttgen, G. (Berlin)—Shah, V.P. (Rockville).

*Session III (Saturday Morning): Models of Percutaneous Absorption*
Krueger, G. (Salt Lake City)—Guy, R. (San Francisco)—Schaefer, H. (CIRD)—Scott, R.C. (Macclesfield)—Wepierre, J. (Chatenay-Malabry).

*Session IV (Saturday Afternoon): Variables in Skin Permeability*
Barry, B. (Bradford)—Cooper, E. (Fort Worth)—Hadgraft, J. (Cardiff)—Finnen, M.J. (Bath)—Täuber, U. (Berlin)—Merk, H. (Köln)—Rougier, A. (Paris).

*Session V (Sunday Morning): Practical Applications of Skin Pharmacokinetics*
Schalla, W. (CIRD)—Peck, C.C. (Bethesda)—Caldwell, J. (London)—Gibson, J.R. (London).

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# British Journal of Dermatology, Volume 115, Supplement 31, August 1986

## CONTENTS

PAGE

Foreword and acknowledgements iii
Experimental evidence that skin carcinogenesis is a multistep phenomenon 1
F.MARKS AND G.FURSTENBERGER
Pharmacological modification of epidermal detoxification systems 9
D.R.BICKERS, M.DAS AND H.MUKHTAR
Defined culture systems for pharmacological and toxicological studies 17
BARBARA A.GILCHREST
Evaluation and relevance of skin sensitivity in animal models 24
A.LOMBARD
The quail uropygial gland for assays of systemic antiandrogen side-effects of topical drugs 33
J.Y.DANIEL, J.H.ABALAIN, Y AMET, S.DI STEFANO, F.BERTHOU, K.TEA, H.H.FLOCH, M.M.BOUTON AND J.SECCHI
Percutaneous absorption of topically-applied antiandrogens: an assessment with three independent methods 36
R.EKERDT, D.OPITZ, H.HUBNER, C.PATZOLD AND M TOPERT
Inhibition of wound healing by antiseptics 41
R.NIEDNER AND E.SCHOPF
The measurement of skin irritation *in vitro* using *ex vivo* rabbit skin 45
M.A.PEMBERTON AND G.J.A.OLIVER
*In vitro* absorption of 1-chloro 2,4-dinitrobenzene (DNCB) through human, hooded rat and mouse epidermis 47
R.C.SCOTT, M.A.THOMPSON, R.J.WARD, J.RAMSEY AND C.RHODES
Effect of repeated skin administration on *in vivo* percutaneous absorption of drugs 49
S.COURTHEOUX, D.PECHENOT, D.A.BUCKS, J.P.MARTY, H I MAIBACH AND J.WEPIERRE
Models for contact sensitization—novel approaches and future developments 53
G.J.A.OLIVER, P A BOTHAM AND I.KIMBER
Short and long-term risks of topical drugs 63
A.ZESCH
Percutaneous absorption of topically-applied drugs in relation to their toxicity 71
H SCHAEFER
Treatment of common and plantar viral warts with human lymphoblastoid interferon-α—pilot studies with intralesional, intramuscular and dermojet injections 76
J.R.GIBSON, S.G.HARVEY, D.KEMMETT, J.SALISBURY AND P.MARKS
Factors influencing anthralin irritancy 80
T.KINGSTON AND N.J.LOWE
Treatment of patients with psoriasis by PUVA therapy and mechlorethamine 82
G MAUDUIT, G.SALLE, A M.SCHOTT AND J THIVOLET
Cutaneous phototoxicity reactions 86
N.J.LOWE
The 'angry back': a new concept or old confusion? 93
A.KLIGMAN AND R.GOLLHAUSEN
Effects of topical corticosteroids on human dermis 101
R.M.LAVKER, N.M.SCHECHTER AND G.S.LAZARUS
Skin toxicity and cellular metabolism: *in vitro* models 108
UWE REICHERT
Endpoints in short-term tests: significance for carcinogenic potential 117
VIRGINIA C.DUNKEL
Three-dimensional culture of human keratinocytes on a dermal equivalent as a model system to study environmental modulation of epidermal physiology *in vitro*: effect of air-exposure 126
D.ASSELINEAU, B.A.BERNARD, C.BAILLY AND Y.M.DARMON
Onc-gene expression in hyperplasia induced by tape stripping or by topical application of TPA 128
P.U.GIACOMONI
The hen's egg test (HET)—an alternative toxicity test 133
N.P.LÜPKE
An evaluation of two approaches for dermatotoxicity testing 136
M A.E MOL, J.VAN GENDEREN AND O.L.WOLTHUIS
Effects of an amphoteric surfactant on the skin barrier: an *in vivo* electro-physiological study 138
G.BORRONI, E.BERARDESCA, P.GABBA, R.PERICOLI, P.T.PUGLIESE, M.BELLOSTA AND G.RABBIOSI
Recent findings limiting immunological and photosensitizing hazards of the tetrachlorodecaoxygen anion complex (TCDO) in wound healing promotion 142
K.W.STAHL AND F.W.KUHNE
Index 149

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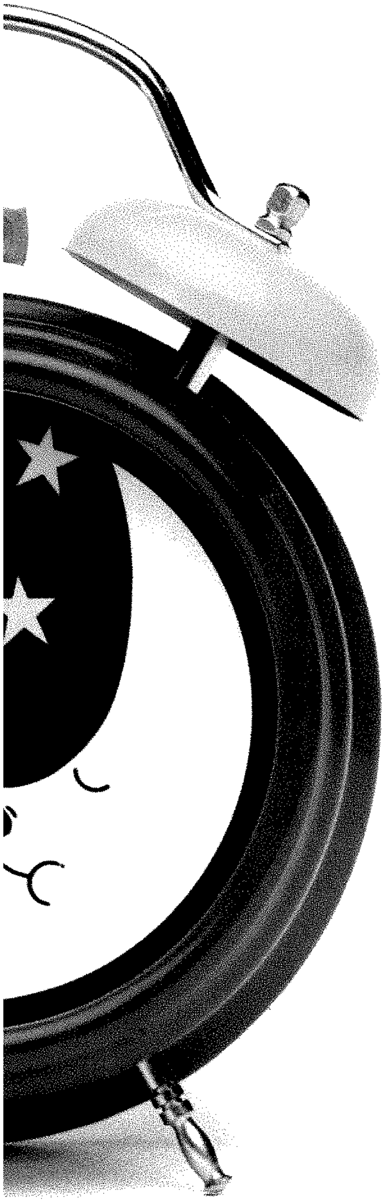

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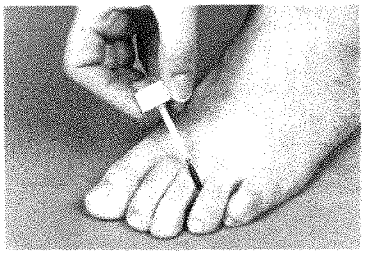

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*Reference*

1 Krebs HA, Wiggins D, Stubbs M, Sols A, Bedoya F. Studies on the mechanism of the anti-fungal action of benzoate. Biochem J 1983 **214**, 657-63

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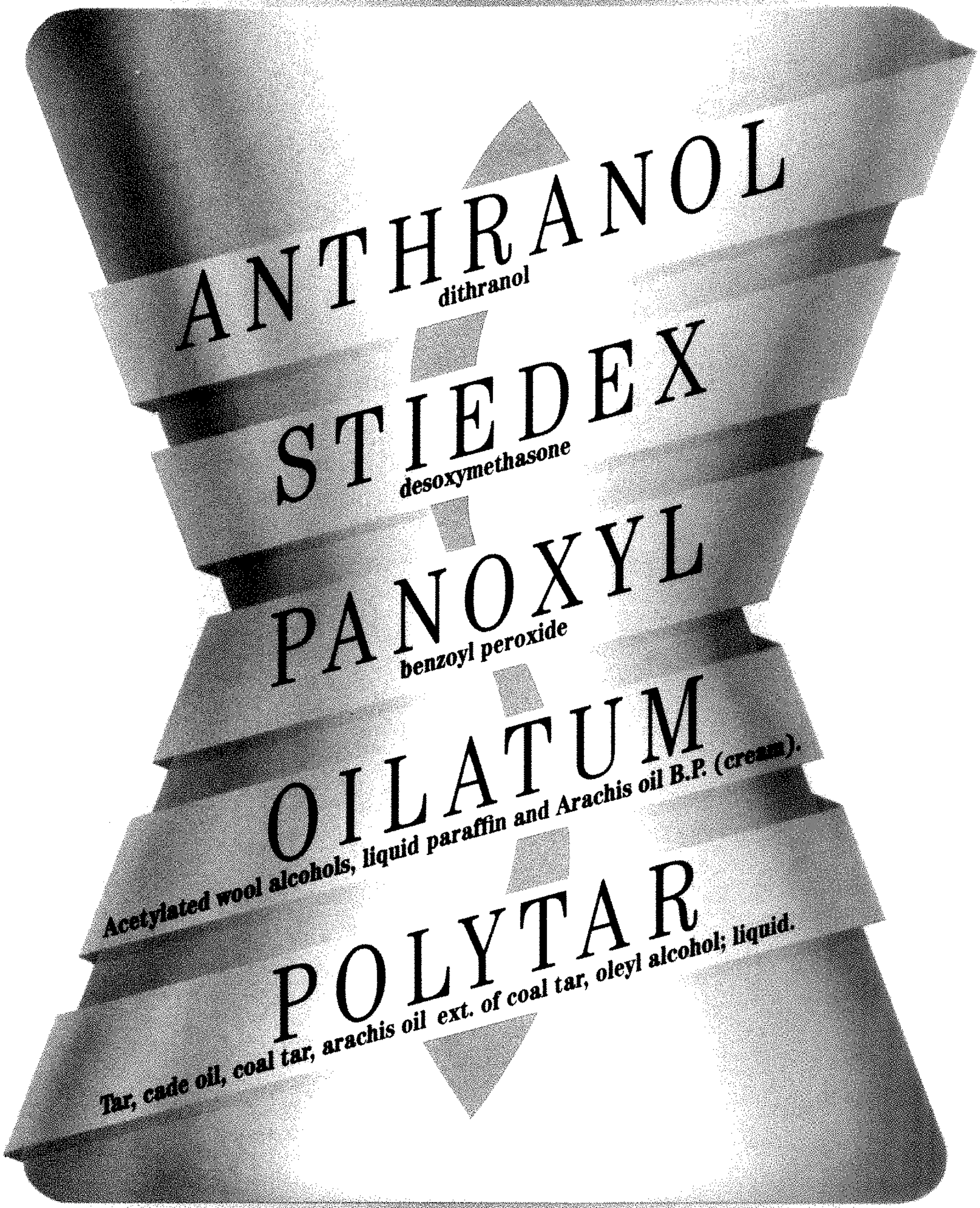

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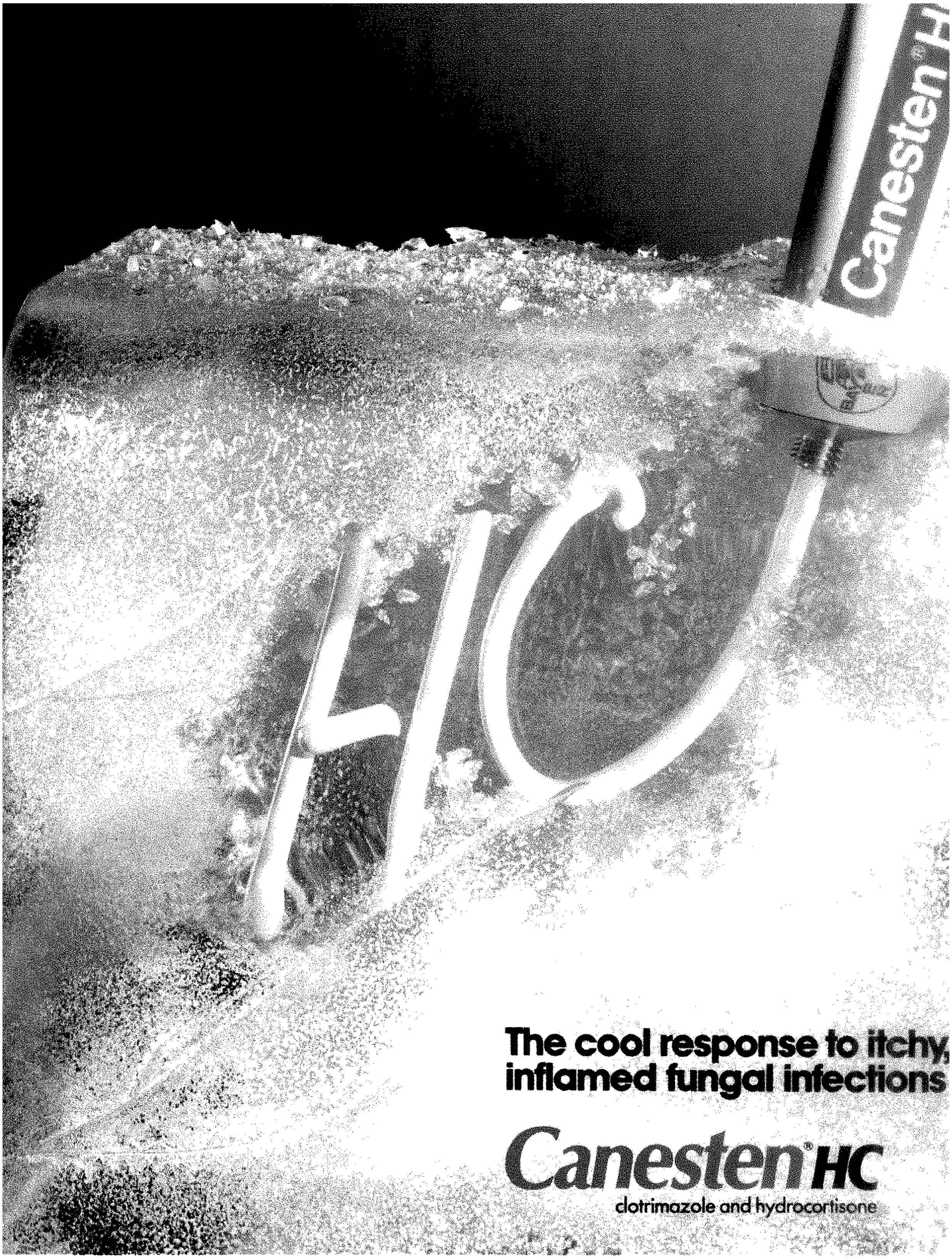
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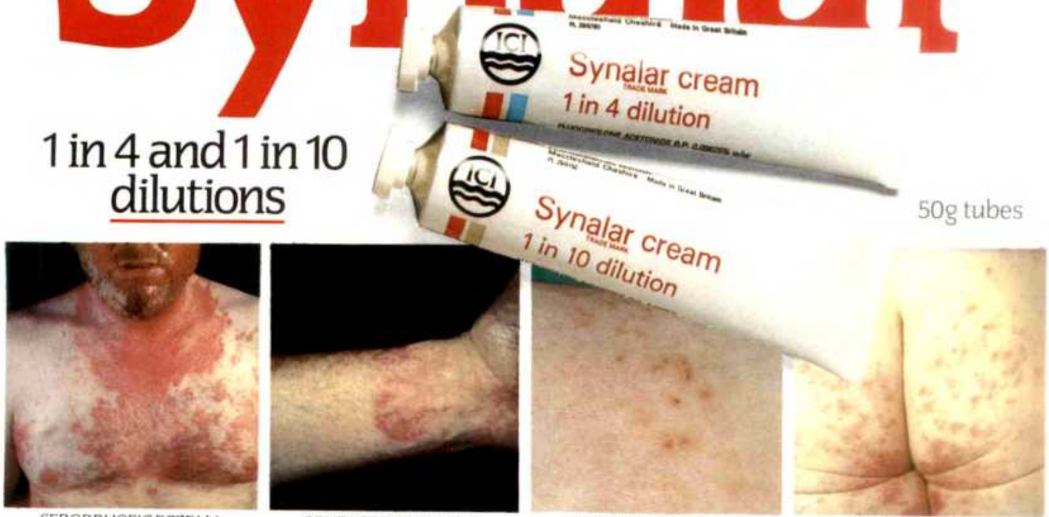

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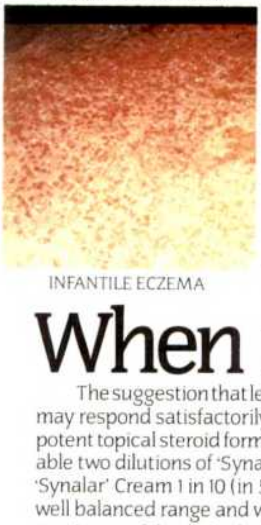

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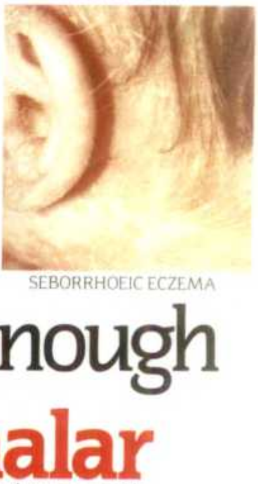

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1985 94 figures IX, 286 pages
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Editors M Goos, E Christophers, University of Kiel

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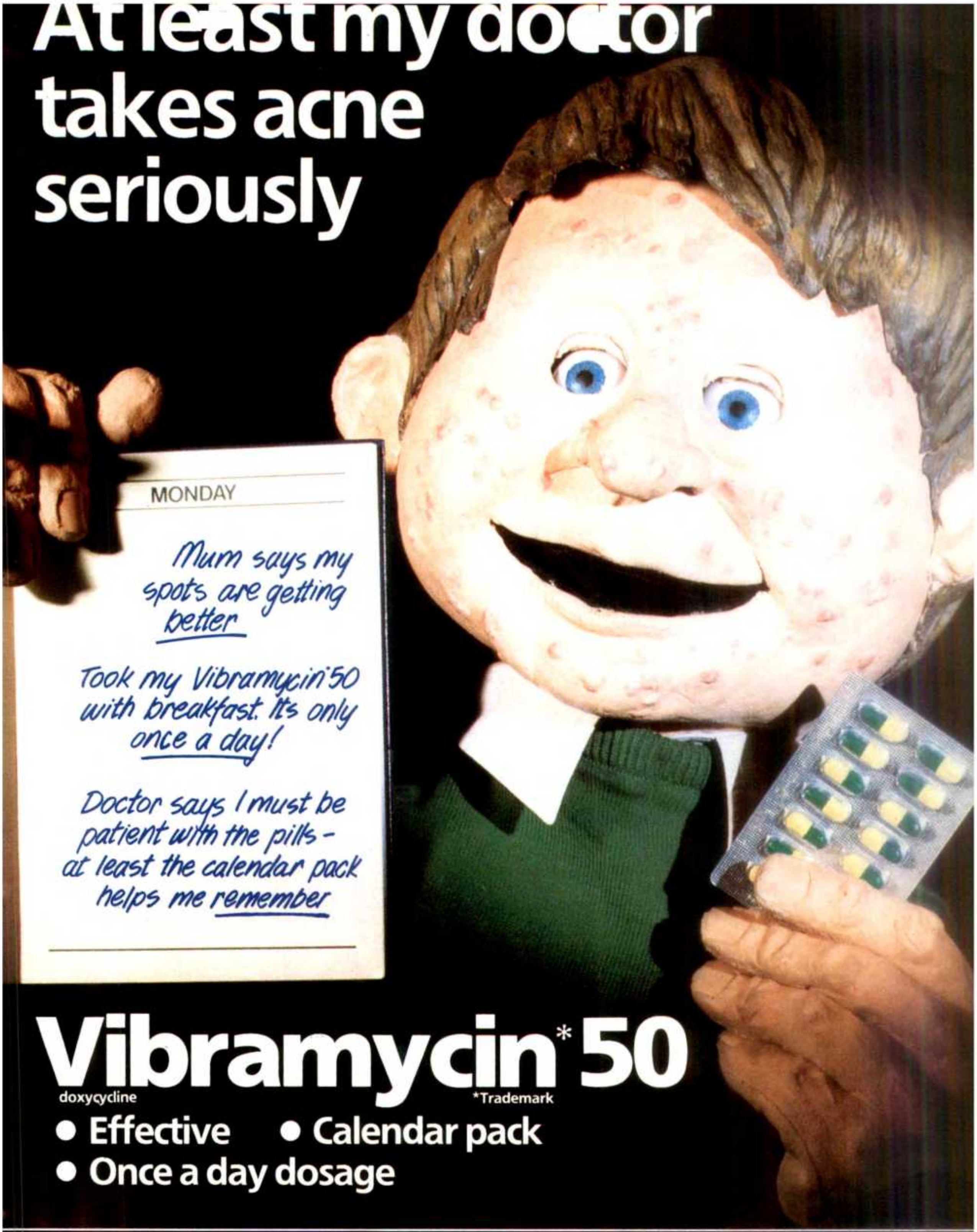
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***Invited Editorial***

# Hidradenitis suppurativa: an androgen-dependent disorder

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The demonstration by Mortimer *et al.* (1986a; this issue, p. 263) that hidradenitis suppurativa responded to treatment with the antiandrogen cyproterone acetate, in a meticulously assessed double-blind trial, substantiates the view that the condition is androgen-dependent. Such a hypothesis was first advanced in the anecdotal report (Ebling *et al.*, 1979) referred to by Mortimer *et al.*, and details of the original four cases are now also published (Sawers, Randall & Ebling, 1986).

Hidradenitis suppurativa is a disorder of the pubic and axillary regions where, in both sexes, the skin is characterized by the presence of terminal hair and functional tubular (so-called apocrine) glands. The pubic and axillary hair is dependent on low levels of androgen for its development. Since apocrine units, often in association with holocrine lipid secreting glands, form hormonally controlled scent organs in many mammals (Ebling, 1977), there are phylogenetic grounds for postulating similar endocrine control—and, indeed, similar odorous function—in man. It is likely that sebaceous as well as apocrine glands are involved in hidradenitis; indeed, Plewig has argued for the inclusion of the hair follicle to form a triad of affected organs (see discussion in Ebling *et al.*, 1979).

Two important questions need to be discussed. The first is whether the disorder results from an abnormally high level of free androgen or whether an enhanced response of the target organs is the critical factor. The second is the extent to which the success of the hormonal therapy can be ascribed to the undoubted lowering of free androgen which it achieves, to blockage of the androgen receptors, or to modification of peripheral testosterone metabolism. Such questions are, of course, of equal moment in considering the causation and treatment of other androgen-dependent disorders.

That hirsutism, alopecia and acne in women can result from over-production of androgen in cases of overt endocrine disturbance is not in doubt. The issue is whether such conditions can be ascribed to abnormally high systemic androgen levels in the absence of obvious disease. Modern techniques for the assay of hormones in body fluids have now made it moderately easy to show that in some patients the level of free testosterone in the plasma is high, within or a little above the normal range. From published data on hirsutism, alopecia and acne, it is usually possible to deduce that the mean total testosterone is significantly higher, or the mean sex hormone binding globulin (SHBG) lower, in patients than in control subjects. If the differences appear undramatic, the calculation of the testosterone/SHBG quotient may sometimes produce a more convincing result. Such demonstrations, carefully analysed and presented, give joy to the investigators. The pattern has, for example been nicely elucidated for acne by Darley *et al.*

(1982), for hirsutism by Lucky *et al.* (1983) and for female diffuse alopecia by Miller *et al.* (1982).

What is striking about all these analyses, however, is not the statistically significant difference between the means for patients and normal subjects, but the large proportion of patients falling within the normal range. Thus, Lucky *et al.* (1983) found that hormone levels were normal in about 40% of women with acne or hirsutism, and Darley *et al.* (1982), who measured prolactin as well as testosterone and SHBG, found 25% to have no abnormality. The outstanding question, therefore, is why such subjects are hirsute or spotty and why an almost infinitely larger number of women of similar hormonal constitution remain unblemished.

Data showing similar trends have recently been published for cases of hidradenitis suppurativa (Mortimer *et al.*, 1986b). The results were perhaps out of tradition, in that no significant abnormality of SHBG was detected, but the median total plasma testosterone in 41 patients with hidradenitis was 1·7 nmol/l as against 1·1 nmol/l in 25 controls, and this difference was highly significant, as was that between the free androgen indices. Nevertheless, no fewer than 32 patients, i.e. 78%, had indices within the normal range, i.e. less than 0·05. Only two showed grossly elevated values, 0·15 and 0·33, respectively; the remaining seven were all between 0·05 and 0·08. The authors were probably right to conclude that the overall pattern of endocrine abnormalities was sufficient to suggest an androgenic basis for the disease. However, in nine patients at most, and perhaps in only two, could the disorder be fairly attributed to an excess of testosterone.

All of the above evidence suggests that the critical factors in most cases of the disorders under consideration must lie in the end organs, rather than in the hormone levels. This view is supported by the finding that in a group of 52 hirsute women neither the rate of sebum excretion on the forehead nor the rate of hair growth on the thigh showed any correlation with either testosterone or SHBG levels (Ebling, Randall & Sawers, 1984). At the same time, sebum excretion was significantly correlated with plasma 5α-dihydrotestosterone (5α-DHT), and hair growth was correlated both with 5α-DHT and with androstenedione.

How should these findings be interpreted? It is known that the urinary excretion of 5α-androstanediol, end product of 5α-reduction of testosterone in target tissues, is abnormally high in both acne and hirsutism (Mauvais-Jarvis, Charranson & Bobas-Masson, 1973). It may be, therefore, that the level of 5α-DHT in the plasma also, to some extent, reflects peripheral metabolism. In respect of androstenedione, however, in spite of the demonstration that it is the major metabolite of testosterone by plucked hairs *in vitro* (Schweikert & Wilson, 1974), it is very unlikely (though perhaps not impossible) that its plasma level reflects the peripheral oxidation of testosterone. More probably, high levels are the basic abnormality, as certainly in gross hormonal disturbances of the adrenal cortex or ovary. This interpretation, however, implies that androgen-sensitive hair follicles respond directly to androstenedione as well as to testosterone.

These thoughts need to be borne in mind in interpreting the therapeutic findings of Mortimer *et al.* (1986a; this issue, p. 263). These authors obtained a clear improvement in five out of 10 patients (Group A) given reverse sequential therapy with 50 mg of cyproterone acetate and 50 μg of ethinyloestradiol for 6 months. In four of these the improvement was maintained when the treatment was changed to Eugynon 50®(E50), containing 500 μg of norgestrel and 50 μg of ethinyloestradiol. Of the remaining five who did not initially respond to CPA, four improved subsequently on E50. Of eight patients treated initially with E50, three improved, and in two of these the improvement was maintained when they were switched to CPA. Of the five who did not respond to E50 initially, two subsequently responded to CPA.

One must sympathize with the dismay of the investigators to discover that both cyproterone acetate and the chosen control regimen of E50 had similar consequences. Nevertheless, in view of the fact that the average duration of the hidradenitis had been 7·6 years, the effectiveness of one or other of the treatments in all but four of 18 cases can hardly be denied.

The authors do not appear to be single-minded in their beliefs about the possible modes of action of the two therapies. On the one hand, in their discussion, they appear to opt for peripheral actions on the grounds that the severity of the hidradenitis did not correlate with the abnormalities of circulating androgens and neither did the response to treatment relate to any reduction in the levels of male hormone. On the other hand, in their summary, they imply that the similar efficacies of CPA and E50 are related to the similar reductions in free androgen index which are induced by the two treatments.

In general, the evidence from all sources supports the view that a peripheral action is the critical factor in the effect of CPA. Firstly, it must be made clear that, while CPA is known to induce modest reductions in both testosterone and androstenedione (Sawers, Randall & Iqbal, 1982), this effect is not unique to the antiandrogen; oestrogens can also produce it. Moreover, CPA does not, when given alone, increase SHBG; the effect of the combined therapy appears to be due entirely to the ethinyloestradiol (Sawers *et al.*, 1982). The evidence that CPA by itself can reduce sebum excretion by about 40% within a few days (Ebling *et al.*, 1979) thus negates the view that its major action is by increasing SHBG concentration and decreasing the free androgen level. In the sebaceous glands at least, the critical action is much more likely to be blockage of the intracellular androgen receptors.

The mode of action of E50 is perhaps more problematical. In the hidradenitis study, the effect on SHBG was not identical with that produced by the reverse sequential therapy with CPA; but the regimens were not identical either, even though they each included ethinyloestradiol. It seems likely that the major action was again peripheral, probably by inhibition of 5α-reductase by the norgestrel.

In summary, reverse sequential therapy with cyproterone acetate and ethinyloestradiol appears to be beneficial in most—though not all—cases of female hidradenitis suppurativa. On these grounds, hidradenitis may be designated as an androgen-dependent disorder and placed alongside acne vulgaris, hirsutism and androgenic alopecia. The finding that, although the mean free androgen index in hidradenitis was greater than normal, most patients had indices within the normal range, does not detract from this conclusion, since such a pattern is characteristic also for the other androgen-dependent conditions. Evidence of several kinds points to the conclusion that, notwithstanding the fact that the level of circulating androgens is reduced by it, antiandrogenic therapy acts principally and critically by blocking the androgen receptors at the target site. It is likely that norgestrel also has a peripheral action, namely inhibition of the metabolism of testosterone to 5α-dihydrotestosterone.

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**Laboratory and Clinical Investigations**

# A double-blind controlled cross-over trial of cyproterone acetate in females with hidradenitis suppurativa

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## SUMMARY

In order to examine whether anti-androgen therapy was effective in hidradenitis suppurativa (HS), ethinyloestradiol 50 μg/cyproterone acetate 50 mg in a reverse sequential regimen was compared with ethinyloestradiol 50 μg/norgestrel 500 μg (Eugynon 50) in 24 female patients. Both treatments produced substantial improvement in disease activity. Seven patients cleared and have remained free of disease for 18 months, five patients improved, four remained unchanged, while two deteriorated. Cyproterone acetate was not clinically significantly more effective than E50, and both gave a similar reduction in free androgen index. Anti-androgen therapy appears to be beneficial in the treatment of hidradenitis suppurativa.

Hidradenitis suppurativa (HS) is a chronic inflammatory disorder of apocrine gland-bearing skin in which deep-seated abscesses and multiple draining sinuses develop (Hurley, 1979). Although infection undoubtedly contributes to the disease (Leach *et al.*, 1979; Highet *et al.*, 1980), the primary event is considered to be poral occlusion (Shelley & Cahn, 1955), with the pathogenesis being similar to that of acne vulgaris. As in acne, androgen action is necessary for the development of HS and significantly elevated plasma testosterone levels have been demonstrated in a group of females with HS (Mortimer *et al.*, 1986).

Cyproterone acetate is of proven benefit in the treatment of acne vulgaris (Fanta, 1980) and hirsutes (Hammerstein, 1979), and, anecdotally, has been shown to be of benefit in HS (Ebling *et al.*, 1979). In view of the similarities between HS and acne vulgaris, a trial of anti-androgen therapy for HS was undertaken. We report here a double-blind, within-patient, controlled trial of ethinyloestradiol 50 μg and cyproterone acetate 50 mg, compared with ethinyloestradiol 50 μg and norgestrel 500 μg (Eugynon 50) in the treatment of HS in 24 female patients, and on the response of circulating androgens to such treatment.

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## METHODS

### *Clinical trial*

*Patients*. Twenty-four females (age range 20–44 years; median 27) with HS were entered into the trial. The diagnosis of HS was based on clinical criteria (Mortimer *et al*., 1986): recurrent boils in apocrine-bearing sites for more than 3 months, presence of comedones in apocrine skin or retroauricular sites and exacerbation of disease pre-menstrually. Disease severity varied from moderate to severe with one to several apocrine sites involved. Duration of disease varied from 6 months to 27 years (median 5 years), with the age of onset ranging from 11 to 43 years (median 18 years). Five patients had axillary disease, six genito-crural involvement, and 11 had both regions affected. Other sites exhibiting HS were mons pubes (1 patient), pre-sternum (1 patient), retro-auricular area (1 patient) and labia majora (1 patient). Patients were considered suitable for the trial if they had no medical contra-indications to hormonal therapy and had HS of at least 6 months' duration. Ethical approval was obtained from the Central Oxford Research Ethics Committee and informed consent was obtained from each patient. Patients were advised to use alternative means of contraception during the trial.

### *Trial design.*

Two treatments were compared in a double-blind cross-over study:

(i) (CPA): ethinyloestradiol 50 μg/cyproterone acetate 50 mg (CPA) in a reverse sequential regimen, where ethinyloestradiol was given on days 5–25 of each menstrual cycle and CPA on days 5–14 of each menstrual cycle (day 1 of the first cycle being the first day of menstrual bleeding).

(ii) (E50): ethinyloestradiol 50 μg/norgestrel 500 μg (Eugynon 50, Schering) given daily on days 5–25 of each menstrual cycle (day 1 of the first cycle being the first day of menstrual bleeding).

Patients were allocated to two groups, matched for age and duration of disease. Group A received CPA followed by E50 and Group B received E50 followed by CPA.

The treatments were given sequentially for a total of 12 months with cross-over at six months. All other medication was stopped at least one month prior to commencing the trial. No other treatment, other than topical antiseptics, was allowed during the trial.

Objective clinical assessment proved difficult owing to the natural fluctuation of the disease activity of HS. Methods using photography, weight of discharge, and spot or boil counting proved unsatisfactory. A system of assessment by the patient over the treatment period as a whole was devised. Three sites, right axilla, left axilla, and the ano-genital region, were assessed independently using three parameters: frequency of lumps and boils, quantity of discharge and pain and discomfort.

Objective assessment was made by one observer (P.S.M.) on the basis of the clinical appearances after each treatment as judged by the number of inflamed and non-inflamed nodules, the degree of induration and tenderness, and the presence of draining sinuses. Comedones, while often found, were not considered an indicator of disease activity. Using a pre-treatment baseline as the reference point, changes in disease activity were scored as clear (+3), much improved (+2), improved (+1), unaltered (0), worse (−1), much worse (−2). Each parameter was scored and an overall global assessment of the response to treatment was based on the sum of the scores for each parameter.

Disease severity was rated by the patients using visual analogue scales, based on an unmarked

10 cm line, at the end of each treatment period, representing 'worst it has ever been' at one end to 'completely healed' at the other.

Patients were reviewed every 3 months unless problems demanded more frequent visits.

### *Androgen assessment*

In an effort to see whether any treatment response was related to alteration in circulating androgens, each patient had blood taken before and on completion of, each 6 month treatment period. Samples were analysed for total testosterone (T), sex hormone binding globulin (SHBG) (measured using a Farmos SHBG-IRMA Kit), dehydroepiandrosterone sulphate (DHEA-S), and prolactin. A Wilcoxon matched pairs signed ranks test was used to compare changes in hormone levels.

## RESULTS

### *Clinical trial*

Eighteen patients completed the trial. Four patients were withdrawn because of treatment side-effects, and two patients because of exacerbation of the disease. Of those completing the trial, 10 patients were in Group A (sequence CPA:E50) and eight patients in Group B (sequence E50:CPA).

Of the 18 patients completing the trial, eight reported minor side-effects while taking E50 and five while taking CPA. E50 produced a variety of non-specific side-effects, while CPA more consistently caused symptoms of weight gain, headaches and breast soreness.

The drop out rate, while relatively high (6 out of 24), was related in four cases to drug intolerance (two to CPA; and two to E50), and in only two cases, to treatment failure.

Objective assessment alone provided insufficient evidence of improvement or deterioration of the disease owing to the fact that the surface appearances in HS frequently belie the events occurring deep down at the base of the apocrine glands. Several conspicuous superficial pustules often proved trivial to the patient, whereas the hidden deep seated solitary abscess with little surface change resulted in severe pain and toxicity. In addition, the natural history of the condition was periods of relative inactivity punctuated by severe exacerbations with one or multiple 'boil' developments. Therefore, an accurate assessment could only be made with knowledge of the frequency and severity of boils and abscesses as judged by the patient. This information was included in the global assessment.

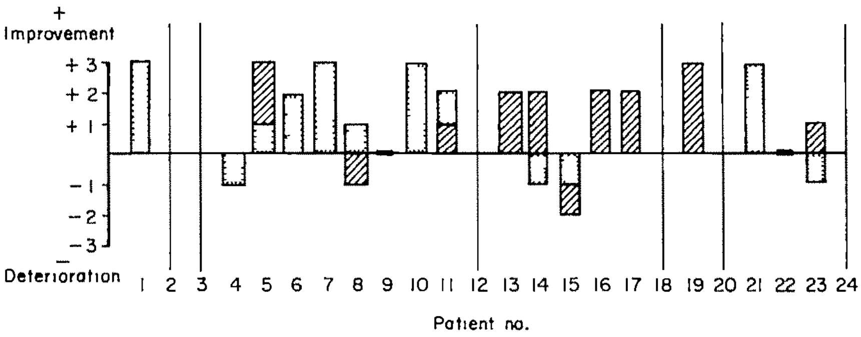

FIGURE 1. Cumulative scores for each patient with each treatment. ■ CPA; ☐ E50; — no change; withdrawn.

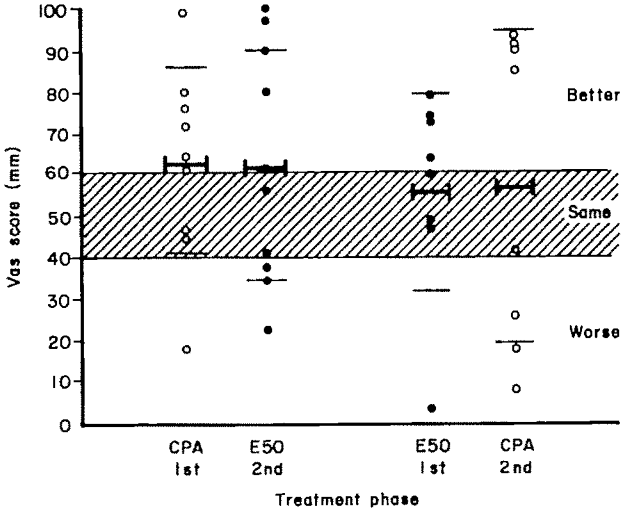

FIGURE 2. Patients' assessment of disease severity rated on visual analogue scales after each treatment. ● E50; ○ CPA. Bars represent mean ± SD.

TABLE 1. Response of circulating androgens to each treatment (mean ± SEM)

| | $n$ | Total testosterone (T) nmol/l | Sex hormone binding globulin (SHBG) nmol/l | Free androgen index T/SHBG |
|---|---|---|---|---|
| Baseline | 17 | 2·12 (±0·26) | 50 (±6·7) | 0·092 (±0·036) |
| Cyproterone acetate (CPA) | 13 | 1·46 (±0·25)** | 159 (±31·6)** | 0·022 (±0·009)** |
| Norgestrel (E50) | 16 | 0·95 (±0·16)** | 69·7 (±8·3)* | 0·017 (±0·004)** |

Comparison with baseline $*P<0{\cdot}05$; $**P<0{\cdot}01$.

The results showed no clinically significant differences between the two treatments as judged by both the disease activity scores (Fig. 1) and visual analogue scales, but there was a striking improvement compared with the baseline with both treatments during the course of the trial; cumulative scores for the first and the second 6 months were +9 in Group A and +4 in Group B.

Five patients improved, four remained the same, two deteriorated. However, most importantly, seven patients cleared, having had continuously troublesome disease for up to 20 years (median 5 years).

The visual analogue scales gave very similar results to the cumulative scores (Fig. 2).

### *Hormone data*

Treatment with E50 produced a significant reduction in the plasma testosterone ($P<0{\cdot}01$)

(Table 1). CPA also reduced the plasma testosterone ($P < 0{\cdot}01$). However, E50 reduced plasma testosterone significantly more than did CPA ($P < 0{\cdot}05$).

Treatment with E50 produced a significant increase in SHBG ($P < 0{\cdot}05$), but this was not as large as that produced by CPA ($P < 0{\cdot}01$). CPA increased SHBG significantly more than did E50 ($P < 0{\cdot}01$).

T/SHBG ratio (free androgen index) was reduced below the baseline values with both treatments ($P < 0{\cdot}01$). There was no statistically significant difference in T/SHBG ratio between the two groups at the end of treatment.

## DISCUSSION

The improvement in the majority of patients by the end of the trial suggested that both E50 and CPA were beneficial in treating HS, and the equal reduction of active circulating androgens in both groups supported this. While an unconcious willingness on the part of both observer and patient to see improvement must be borne in mind, this cannot possibly explain the clearance in seven patients, nor the striking improvement in another five. When choosing a suitable placebo in a double-blind trial with CPA, an alternative hormonal preparation had to be considered and to use less than 50 μg ethinyloestradiol with high dose CPA would have been inappropriate. E50 is anti-androgenic, as circulating androgen levels demonstrated, and this therefore minimized or negated the demonstration of any anti-androgenic benefit from CPA.

Androgens are only one aspect of a multifactorial aetiology in HS (Hurley, 1979; Shelley & Cahn, 1955). Infection plays a significant role in established disease but is probably not of primary importance in the pathogenesis of the disease. Anti-androgen therapy alone was less effective in those cases complicated by infection with pathogenic bacteria, e.g. *Staph. aureus*. Conversely, it was noticeable that all patients who cleared had negative bacteriology (bacteriological study was not part of the protocol and therefore not performed in all patients). Chronicity of disease was not a reason for lack of response to treatment, although, in general, patients with more extensive disease in terms of fibrosis or scarring, open cavities and deep seated loculated abscesses, fared less well.

HS patients are a heterogenous group endocrinologically, many possessing other features of cutaneous virilism, e.g. hirsutes, and acne vulgaris with or without elevated circulating androgens (Mortimer *et al.*, 1986). As with acne, severity of HS does not seem to correlate with abnomalities in circulating androgens, nor does treatment response seem to relate to any reduction in male hormone levels. Such parameters are therefore only a crude indicator of an androgenic abnormality, which probably exists within the tissues of the target organ, in this case the apocrine gland.

There is evidence to support the view that HS is an androgen-dependent condition and benefits from anti-androgen therapy. The similarities between acne vulgaris and HS in their basic pathogenesis, and tendency to co-exist support this. However, HS differs from acne in site and its complication by infection. Anti-androgen therapy is of most benefit in disease not complicated by pathogenic bacteria and therefore may ultimately be most useful in combination with other therapies, e.g. antibiotics, or after surgery to prevent relapse. In general, HS is only recognized at a moderately advanced stage, and therefore to secure adequate control of the disease, early diagnosis and prompt treatment is crucial.

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# Control of hidradenitis suppurativa in women using combined antiandrogen (cyproterone acetate) and oestrogen therapy

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## SUMMARY

The effects of combined treatment with the antiandrogen, cyproterone acetate, and ethinyl oestradiol on four women with long-standing hidradenitis suppurativa have been investigated. The condition was controlled successfully in all patients with 100 mg/day cyproterone acetate using the reversed sequential regimen; lowering the antiandrogen to 50 mg/day caused deterioration. Before treatment, plasma testosterone levels were within the normal range, but plasma androstenedione values were raised and sex hormone binding globulin levels were low. On treatment, the androstenedione concentration fell and sex hormone-binding globulin values were raised. However, since these levels were unaltered by reducing the antiandrogen dosage, the main action of the therapy is probably that of the antiandrogen within the target cells.

Hidradenitis suppurativa, or apocrine acne, is thought to occur as a result of closure of the apocrine pore with subsequent bacterial infection (Shelley & Cahn, 1955); androgens have been implicated in its aetiology (see, for example, Brunsting, 1952).

Since acne, an androgen-dependent condition also involving pore occlusion and bacterial infection, responds to combined cyproterone acetate and oestrogen therapy (Neumann, Aydinlik & Lachnit-Fixson, 1984), we have investigated the effect of such treatment on four women with long-standing hidradenitis suppurativa. Although the antiandrogen is the most effective component of such treatment for hirsutism (Sawers, Randall & Iqbal, 1982), cyproterone acetate must be given to women of reproductive age in conjuction with oestrogen to ensure contraception so that the possible feminization of a male foetus is avoided.

## METHODS

### *Patient histories*

All four women had at least one child and none was hirsute.

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*Patient 1.* Aged 33 years at the start of treatment, this patient had had recurrent vulval and pubic abscesses for 5 years, particularly during the week before, and after, menstruation. Previous treatment had included antibiotics and radiotherapy.

*Patient 2.* Aged 39 years, this patient presented with a history of boils in the groin since childhood, and in the axilla for 13 years. A pilonidal sinus had been excised 10 years earlier and she had previously been prescribed numerous courses of antibiotics.

*Patient 3.* Aged 29 years, this patient had had lesions in the vulva and external auditory meatus since the age of 10 years. She had also developed severe facial acne over the previous 3 years. Her earlier treatment had included plastic surgery of the groin and 6 years on a contraceptive pill (Ovran).

*Patient 4.* Aged 24 years, this patient presented with pustules on the vulva which had persisted for 5 years, becoming increasingly painful in the 6 months before treatment commenced. She had taken a contraceptive pill (Ovranette), but had ceased this prior to attending the clinic.

### *Clinical protocol*

Patients attended clinics at 28-day intervals where, in addition to clinical investigation, sebum production was measured by a modification of the method of Strauss and Pochi (1961) as previously described by Ebling and Randall (1985), and blood samples were taken for various hormonal measurements. The endocrine assays have been reported by Sawers, Randall & Iqbal (1982). At least two basal measurements were obtained before treatment commenced. Clinical improvement was assessed both subjectively from patients' observations and objectively by recording the severity and location of the condition assessed by the same clinician; photographs were also taken, both before and during treatment.

All patients started on the reversed sequential regimen of Hammerstein and Cupceancu (1969) with 100 mg of cyproterone acetate per day for 10 days and 50 $\mu$g of ethinyl oestradiol per day for 21 days. After 3–7 months, the oestrogen component was reduced to, and maintained at, 30 $\mu$g per day to reduce potential side-effects of oestrogen treatment; the cyproterone acetate was also reduced to 50 mg per day in all patients at some time during their therapy. The levels for individual patients were altered at different stages during their treatment for various clinical reasons.

## RESULTS

### *Clinical results*

All patients exhibited rapid objective clinical improvement and also reported a subjective impression of improvement after only one to two cycles of treatment. This improvement increased and was then maintained during therapy (Fig. 1). The facial acne in Patient 3 also improved rapidly.

When cyproterone acetate was reduced to 50 mg per day three patients showed worsening of their symptoms. Patient 1 also noted recrudescence during the parts of the cycle where cyproterone acetate was not taken; this patient also had two periods, of 3 and 4 months respectively, taking Euguynon 30 (i.e. oestrogen but no antiandrogen), but on both occasions her abscesses returned.

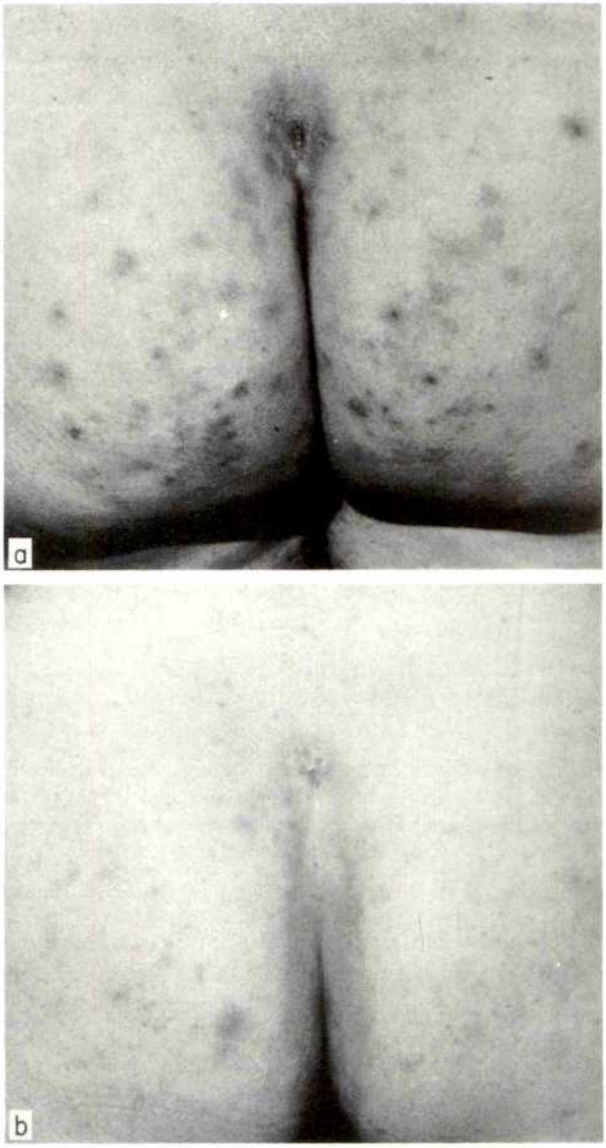

FIGURE 1. The effects of treatment with cyproterone acetate and oestrogen. (a) before treatment; (b) after 6 months of therapy.

Patients 3 and 4 showed no recurrence after treatment had been discontinued for 14 and 5 months respectively, after 23 and 11 cycles of therapy. Patient 4 had shown recurrence previously during a 6-month withdrawal from treatment after an accident, when she had already completed eight cycles.

### *Sebum production*

Sebum production in Patients 1 and 3 was initially high, but was rapidly reduced to normal female levels after one to two cycles of treatment and remained low (Fig. 2). The other women had normal sebum production rates both before, and during, therapy.

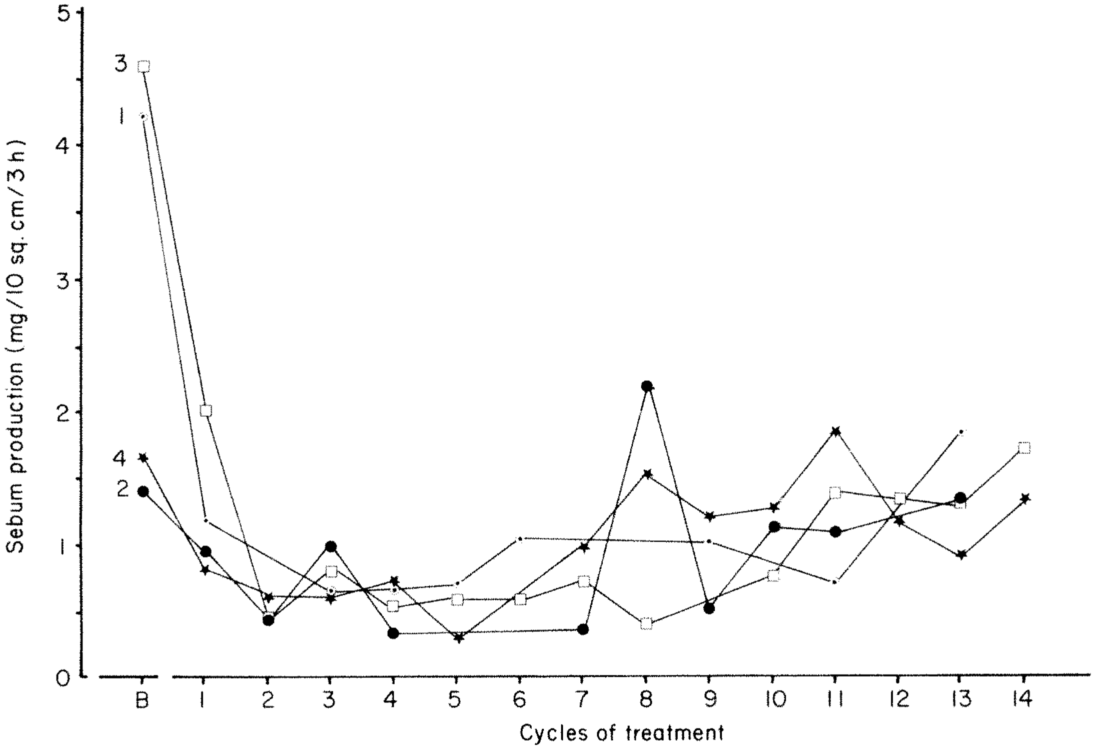

FIGURE 2. Sebum production rates on the forehead of four women with hidradenitis suppurativa before, and during, treatment with cyproterone acetate and ethinyl oestradiol. ⊙ Patient 1; ● Patient 2; □ Patient 3; ★ Patient 4. Patient 3 was taking a contraceptive pill prior to treatment.

### *Endocrine data*

Although plasma testosterone levels were within the normal range, values for androstenedione, the main circulating androgen in women, were high, except in Patient 3 who was taking a contraceptive pill (Table 1). The major plasma binding protein for sex steroids, sex hormone-binding globulin, was present in concentrations well below the normal female levels, except in Patient 3.

On treatment, plasma testosterone levels remained within the normal range, while androstenedione concentrations were reduced to around the upper limit of the normal range. Sex hormone-binding globulin levels were greatly increased to 100–240 nmol DHT bound per litre; levels in Patient 3 were also increased to about 200 nmol. This means that the ratio of testosterone:sex hormone-binding globulin was rapidly lowered.

There was no consistent effect on any of the endocrine parameters during alteration of the doses of either cyproterone acetate or oestrogen.

### *Side-effects*

All four patients reported feeling depressed during treatment, but three had severely ill parents or suffered a bereavement during the period of study. One patient (Patient 2) withdrew from therapy after 13 months due to depression; this patient had a previous history of depression.

Patient 1 reported breast tenderness and Patient 3 became amenorrhoeic when treatment was discontinued. No consistent effects on weight were noted.

TABLE 1. Hormone levels in four women with hidradenitis suppurativa

| | Plasma hormones | | Sex hormone-binding globulin | Testosterone/sex hormone-binding globulin |
|---|---|---|---|---|
| Patient | Testosterone (nmol/l) | Androstenedione (nmol/l) | (nmol DHT/l) | |
| 1 | 0·92 | 10·45 | 19·0 | 0·050 |
| 2 | 1·10 | 13·20 | 18·2 | 0·059 |
| 4 | 0·80 | 11·37 | 30·6 | 0·030 |
| Mean | 0·94 | 11·67 | 22·8 | 0·046 |
| 3* | 0·48 | 5·47 | 59·0 | 0·008 |
| Normal women | < 2·8 | < 8·0 | 58·6 ± 1·01 | — |
| Hirsute women | 1·53 ± 0·46 | 11·09 ± 3·53 | 30·29 ± 14·41 | 0·069 ± 0·050 |

Normal values – mean ± SD obtained from 59 women aged between 17 and 45 years (mean 31 years) and values for hirsute women (mean ± SD) from our parallel studies on 54 women with hirsutism. * Patient 3 was taking a contraceptive pill.

## DISCUSSION

The combined cyproterone acetate (100 mg per day) and oestrogen treatment was very effective in controlling the long-standing hidradenitis suppurativa in all four patients. This confirmed our earlier report of a single patient (Ebling *et al.*, 1980), which has since stimulated a larger study of women with hidradenitis suppurativa and their treatment with cyproterone acetate (Mortimer *et al.*, 1986a,b). In two cases the improvement was maintained for several months after treatment had ceased.

The finding that plasma testosterone levels were within the normal range while androstenedione levels were raised contrasts with the raised testosterone levels reported by Mortimer *et al.* (1986a) in both hidradenitis suppurativa and hirsute patients; they did not measure androstenedione levels. Although this difference could be due to our much smaller number of subjects, the results of our large study of hirsute women also showed raised androstenedione and normal testosterone levels in accordance with other published data (Sawers, Randall & Iqbal, 1982). Sex hormone-binding globulin was below normal female levels, except in Patient 3. Exogenous oestrogen from the contraceptive pill is known to raise sex hormone-binding globulin concentration (Sawers, Randall & Iqbal, 1982). When sex hormone-binding globulin levels are low, it is generally believed that 'free' testosterone, i.e. that which is not bound to the carrier protein and is therefore more readily available to the cell, is increased. The ratio of testosterone to sex hormone-binding globulin can be used as an indicator of 'free' testosterone; in these patients, with the exception of Patient 3, the ratio was high, similar to hirsute patients (Table 1).

Although some side-effects were noted, these were tolerable to most patients. Depression is difficult to evaluate and in most of our patients coincided with traumatic events in their lives; the single patient who withdrew from treatment had a previous history of depression. The side-effects in patients taking combined cyroterone acetate and oestrogen therapy reported by other workers are similar to those of oral contraceptives containing 50 µg of ethinyl oestradiol (see review by Moltz, Schwartz & Hammerstein, 1982).

The possible sites of action of the treatment include: the competitive inhibition of the androgen receptor by the antiandrogen in the target cells; decreased androgen production caused by negative feedback at the pituitary; and increased sex hormone-binding globulin levels, due to the oestrogen component, reducing the testosterone readily available to the cells. Although the circulating levels of androstenedione were lowered and those of sex hormone-binding globulin were raised, the main effect appears to be due to the local action of the cyproterone acetate in the skin, since the endocrine changes which occurred during treatment were not altered by reducing the antiandrogen dosage to 50 mg per day, but the patients' condition deteriorated. In addition, one patient, who was already taking a contraceptive pill prior to this therapy, showed only limited endocrine changes but dramatic clinical improvement of her facial acne and hidradenitis suppurativa.

These results lend support to the concept of hidradenitis suppurativa as an androgen-dependent disorder. They also suggest that androgen-dependent skin disorders, such as acne and hirsutism, are mainly caused by abnormal target organ responses to androgens, since our patients generally showed endocrine similarities to hirsute women before treatment, but were not hirsute.

Although combined cyproterone acetate and oestrogen therapy should not be used for women with a history of thromboembolic diseases because of the increased blood coagulability caused by oestrogens (Moltz, Schwartz & Hammerstein, 1982), the treatment seems highly appropriate for female patients with hidradenitis suppurativa. We have found that a dosage of 100 mg per day is necessary for full control of the condition.

## ACKNOWLEDGMENTS

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# Photosensitivity due to retinoids: clinical and laboratory studies

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## SUMMARY

Six subjects taking isotretinoin were studied, none of whom had clinical or phototest evidence of photosensitivity. Of nine subjects taking etretinate, one had convincing clinical photosensitivity consisting of a burning erythema on sunlight exposure. His phototesting results showed a marked abnormality from 300 ± 5 nm to 365 ± 30 nm which returned close to normal limits within one month of stopping therapy. Although clinically normal, another subject taking etretinate had similar phototest evidence of abnormal photosensitivity. *In vitro* photohaemolysis studies demonstrated that tretinoin (all-*trans*-retinoic acid) and isotretinoin have a phototoxic potential, while etretinate has none. However, the major metabolite of etretinate (Ro 10-1670) had a phototoxic potential greater than that of tretinoin. The apparently low incidence of photosensitivity suggests that an idiosyncracy is responsible, perhaps due to a disorder of pharmacokinetics or metabolism. Clinical cases should use appropriate photoprotection against UVB and UVA wavebands.

The synthetic retinoids etretinate (Tigason®) and isotretinoin (Roaccutane®) have established roles now in dermatological therapy (Dicken, 1984; Orfanos, 1985). As with all-*trans*-retinoic acid (Pedace & Stoughton, 1971; Papa, 1975), in addition to a number of more common well-documented side-effects, there is a possibility that these drugs induce an abnormal cutaneous photosensitivity. With isotretinoin, while photosensitivity is listed frequently as an adverse effect, reports in the literature are contradictory. An increased susceptibility to sunburn reactions has been stated to occur in 5–12% of patients (Angel, 1983; Strauss *et al.*, 1984), yet the work of others has failed to detect such a problem (Diffey, Spiro & Hindson, 1985). A single case report of clinical photosensitivity by McCormack and Turner (1983) described burning erythema of sun exposed sites which was thought to be phototoxic in origin. Phototesting a group of subjects taking isotretionoin proved negative (Diffey *et al.*, 1985).

Etretinate has also been reported as causing sunburn in 7–9% of patients (Grekin, Ellis & Vorhees, 1984). Two early phototesting studies failed to produce evidence of an abnormal

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response in subjects taking the drug (Ippen, Hofbauer & Schauder, 1978; Goerz & Orfanos, 1978). There is no published evidence for a photosensitizing action of the retinoids *in vitro*.

We report here the results of clinical studies of the possible photosensitivity accompanying treatment with either etretinate or isotretinoin, and laboratory studies to investigate the phototoxic potential of isotretinoin, of etretinate, of Ro 10-1670, the major metabolite of etretinate and of all-*trans*-retinoic acid.

## METHODS

### *Subjects*

Nine subjects, seven with psoriasis, one with ichthyosis (non-bullous ichthyosiform erythroderma) and one with pityriasis rubra pilaris, taking etretinate (10–50 mg/day) for 3–12 months, were studied (Table 1).

Six subjects with acne, taking isotretinoin (10–30 mg/day) for 4–24 weeks, were also investigated (Table 2).

TABLE 1. Clinical features of subjects taking etretinate

| Subject | Sex | Age (years) | Skin type | Diagnosis | Daily dosage (mg) | Duration of treatment (months) | Clinical photosensitivity |
|---|---|---|---|---|---|---|---|
| 1 | F | 41 | I | Psoriasis | 30 | 7 | No |
| 2 | M | 57 | IV | Psoriasis | 30 | 6 | No |
| 3 | M | 57 | I | Psoriasis | 10 | 4 | No |
| 4 | M | 63 | I | Pityriasis rubra pilaris | 30 | 11 | No |
| 5 | M | 69 | IV | Psoriasis | 25 | 6 | No |
| 6 | M | 60 | II | Psoriasis | 50 | 9 | No |
| 7 | F | 42 | II | Psoriasis | 50 | 3 | No |
| 8 | F | 41 | II | Psoriasis | 50 | 12 | No |
| 9 | M | 31 | I | Ichthyosis* | 50 | 3 | Burning erythema |

* Non-bullous ichthyosiform erythroderma.

TABLE 2. Clinical features of subjects with acne vulgaris taking isotretinoin

| Subject | Sex | Age (years) | Skin type | Daily dosage (mg) | Duration of treatment (weeks) | Clinical photosensitivity |
|---|---|---|---|---|---|---|
| 1 | M | 24 | III | 30 | 10 | No |
| 2 | M | 20 | II | 10 | 12 | No |
| 3 | M | 19 | III | 10 | 8 | No |
| 4 | M | 19 | II | 30 | 24 | No |
| 5 | M | 22 | II | 20 | 4 | No |
| 6 | M | 22 | III | 40 | 7 | No |

None of the subjects showed signs of milia, onycholysis, or abnormalities of pigmentation.

All subjects were questioned and examined for features of photosensitivity. None of the subjects was taking any other systemic medication and none had a previous history of a photodermatosis.

### In vivo *studies*

Phototesting studies were carried out on the skin of the upper back using a 150 W xenon arc solar simulator (Johnson & Mackenzie, 1982), filtered to provide whole solar spectrum radiation (> 280 nm) or whole spectrum minus UVB (> 315 nm) to obtain a preliminary indication of abnormal photosensitivity, and a diffraction grating monochromator with a 1·6 kW xenon arc source to provide various wavebands in the UV and visible spectrum (Mackenzie & Frain-Bell, 1973) to obtain more clearly defined action spectra. Minimal erythema dose (MED) determinations were performed by exposing skin areas, 8 mm in diameter, to a succession of radiation doses selected from a geometric series with incremental steps of approximately 20%. Any erythema reactions were recorded immediately after the exposures, continuously during the first hour and thereafter at 2, 4, 6, and 24 h, some subjects also being examined at 48 h.

### In vitro *studies*

Two model systems for phototoxicity were used: (i) *Candida albicans* (Daniels, 1965; Kavli & Volden, 1984). Retinoids in solid form or applied in solution to filter paper discs and then dried, were placed on Sabouraud's agar plates freshly seeded with *C. albicans*. Duplicate plates were set up, one being maintained in the dark as a control and the other exposed to UVA radiation (315–400 nm) from an array of fluorescent tubes (Philips TL20W/08 F20T12 BLB) at an irradiance of 0·2 mW/cm$^2$ for 48 h. A clear zone around the potential photosensitizer in the irradiated plate, and complete growth in the dark control, was considered indicative of phototoxicity. (ii) Photohaemolysis (Hetherington & Johnson, 1984). The retinoids were dissolved first in dimethylsulphoxide and then added in $\mu$l amounts to a dilute suspension of washed red blood cells (RBC) (1 in 1000) in barbitone buffered normal saline at pH 7·2 to provide various final concentrations of the drugs. After 30 min equilibration in the dark, 5 ml aliquots, providing monolayers of RBCs in 5 cm diameter plastic petri dishes (Sterilin), were exposed to a standard 11·52 J/cm$^2$ dose of UVA from an array of Sylvania (FR74T/PUVA) fluorescent tubes. Control preparations were maintained in the dark. To examine whether any photohaemolysis obtained might be due to a toxic photoproduct of the retinoids, an extra control consisted of retinoid solutions irradiated with UVA and then added to the RBC suspension. Following the exposure and a 30 min equilibration in the dark, the suspensions were centrifuged at 2000 rpm, and the amount of haemoglobin in the supernatant was determined as cyanmethaemoglobin using Drabkin's reagent, reading the optical density at 420 nm against a blank of buffered saline and Drabkin's reagent. A 100% haemolysis value was obtained from a 1 in 1000 dilution of RBCs in distilled water.

At the concentrations used, the retinoids plus Drabkin's reagent produced an optical density at 420 nm greater than the blank. This absorbance was lost to varying degrees when the retinoids were exposed to UVA. Extra controls were therefore set up consisting of retinoid solutions irradiated with UVA, and retinoid solutions maintained in the dark.

## RESULTS

Of the nine subjects taking etretinate, one (Subject 9, Table 1) who had a tendency to sunburn without tanning (skin type I) before taking the drug, noted a worsening of the skin response to sunlight in that within 3 weeks of starting etretinate (1 mg/kg) he developed a recurrent, uncomfortable burning erythema in skin exposed to direct sunlight. Within 2 weeks of stopping the medication, his skin response to sunlight returned to normal. The other eight subjects had no abnormal photosensitivity. For the six subjects taking isotretinoin, clinical histories failed to produce any evidence of increased susceptibility to sunburn or other abnormal responses to sunlight (Table 2).

### In vivo *studies*

The results of the phototesting studies were compared with those of a known normal population. Of the nine subjects taking etretinate, six responded normally while two (Subjects 8 and 9) produced abnormal delayed erythema responses, in that the MEDs for whole spectrum solar simulator radiation were lower than normal (Table 3). The MEDs for whole spectrum minus the UVB were normal, indicating that the abnormal photosensitivity involved the UVB wavelengths. For these two subjects monochromator testing produced lower than normal MEDs for wavelengths from 295 ± 5 nm through 365 ± 30 nm, with the increased sensitivity most pronounced between 305 and 320 nm (Table 3). Subject 9 stopped taking etretinate and one month later phototesting results were normal apart from a persistent sensitivity at 335 ± 30 nm

One subject (Subject 3) produced evidence for abnormal photosensitivity in the form of an immediate erythema reaction to 365 ± 30 nm (12·0 J/cm$^2$) and 400 ± 30 nm (47 J/cm$^2$) which persisted for longer than 5 min, a reaction not seen with these doses in the normal population.

The phototesting results for five of the six subjects taking isotretinoin were all within normal limits (Table 3). One subject (Subject 3) produced reactions at the lowest level of the normal range for wavelengths 310 and 315 nm and below this at 320 nm. However, this is not a striking example of abnormal photosensitivity.

### In vitro *studies*

*Candida albicans.* None of the retinoids tested was found to be phototoxic.

*Photohaemolysis.* Because the retinoids themselves interfered with the optical density measurements in varying ways depending on concentration and irradiation, the % haemolysis obtained was calculated from the formula:

$$\%\ \text{haemolysis} = \frac{\text{OD RBC supernatant} - \text{OD retinoid solution}}{\text{OD } 100\%\ \text{haemolysis}} \times 100$$

When this procedure was used, it was evident that at the concentrations used there was no effect of the retinoids alone. Etretinate was not phototoxic, but all-*trans*-retinoic acid, Ro 10-1670, and isotretinoin were phototoxic at relatively high concentrations (Table 4). No haemolysis was obtained with pre-irradiated retinoid solutions.

Under the conditions of these experiments the relative phototoxicity of the retinoids may be expressed in terms of the concentrations required to produce 50% haemolysis (Table 5). The major metabolite of etretinate had the greatest phototoxic potential, marginally higher than that of all-*trans*-retinoic acid, and both these are more phototoxic than isotretinoin by factors of 8 and 5 respectively.

TABLE 3. Minimal erythema dose (MED) determinations for abnormal delayed reactions in subjects taking retinoids

| | Solar simulator | | Monochromator | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Waveband (J/cm$^2$) | | Waveband ± 5 nm (mJ/cm$^2$) | | | | | | Waveband ± 30 nm (J/cm$^2$) | | | | |
| | > 280 nm | > 315 nm | 295 | 300 | 305 | 310 | 315 | 320 | 330 | 335 | 365 | 400 | 430 |
| Etretinate | | | | | | | | | | | | | |
| Subject 8 | 4* | 100 | 3·3* | 1·5* | 1·5* | 3·3* | 15* | 33* | 220* | 0·82* | 6·8* | > 47 | > 82 |
| Subject 9 | 2* | 100 | 6·8 | 3·9* | 1·5* | 3·9* | 68* | 180* | 1200* | 1·8* | 4·7* | > 47 | > 82 |
| Subject 9a** | NT | NT | 10 | NT | > 19 | NT | NT | 1200 | NT | 1·5* | > 10 | > 47 | NT |
| Isotretinoin | | | | | | | | | | | | | |
| Subject 3 | 10 | > 100 | > 8·2 | 18 | > 33 | 150 | 560 | 680* | > 2200 | > 2·2 | > 10 | > 47 | > 82 |
| Lowest MED in normals | 7 | 100 | 6·8 | 15 | 22 | 150 | 560 | 820 | 2200 | 2·2 | 8·2 | > 47 | > 82 |

NT: Not tested.

* Abnormal response dose.

** Subject 9a was Subject 9 tested again one month after stopping etretinate treatment.

For Subjects 1–7 taking etretinate, and Subjects 1, 2, 4, 5 and 6 taking isotretinoin, the MEDs at each waveband were equal to or greater by 1 or more steps in the exposure series than the lowest normal shown here.

TABLE 4. Photohaemolysis with all-*trans*-retinoic acid, isotretinoin, etretinate, and Ro 10-1670, the major metabolite of etretinate

| Concentration μg% | Haemolysis mean % ± s.d. | No. of samples |
|---|---|---|
| All-*trans*-retinoic acid | | |
| 74 | 7·50 | 2 |
| 184 | 18·25 ± 8 5 | 8 |
| 368 | 41·00 ± 11·3 | 10 |
| 736 | 77·00 ± 19·8 | 10 |
| 1104 | 98·00 ± 3·1 | 4 |
| 1472 | 100 | 2 |
| 1840 | 100 | 2 |
| Dark haemolysis 0% up to 1840 μg% | | |
| Isotretinoin | | |
| 184 | 0 | 2 |
| 368 | 6·30 ± 2·7 | 6 |
| 736 | 8·60 ± 3 7 | 10 |
| 1472 | 29·00 ± 18·7 | 6 |
| 3680 | 83·00 ± 24 2 | 8 |
| 7360 | 73·00 ± 15 0 | 6 |
| Dark heamolysis 0% up to 7360 μg% | | |
| Etretinate | | |
| Photohaemolysis and dark haemolysis 0% from 184–7360 μg% | | |
| Ro 10-1670 | | |
| 36 8 | 6·50 | 2 |
| 92 0 | 8·75 ± 1·9 | 4 |
| 184 | 23·25 ± 14·0 | 8 |
| 368 | 65·75 ± 18 5 | 8 |
| 736 | 98 0 | 2 |
| 920 | 100 | 4 |
| 1840 | 100 | 4 |
| Dark haemolysis 0% up to 3680 μg% | | |

TABLE 5. Photohaemolysis with retinoids. Concentration of retinoids required for 50% haemolysis with 11 52 J/cm$^2$ UVA ($HD_{50}$)

| Retinoid | $HD_{50}$ (μg%) |
|---|---|
| Etretinate metabolite<br>(Ro 10-1670) | 300 |
| Retinoic acid | 460 |
| Isotretinoin | 2300 |
| Etretinate | no haemolysis |

## DISCUSSION

Although the results of this study confirm previous findings that abnormal cutaneous photosensitivity due to systemic retinoids is rare, we have obtained evidence on clinical grounds and with controlled phototesting that it does occur and, using the photohaemolysis model, we have shown that these compounds or their metabolites, do have a phototoxic potential.

The case of etretinate-induced photosensitivity (Subject 9) which was accompanied by positive phototest results from 300 ± 5 nm to 365 ± 30 nm with virtual clearance after stopping the drug, is strong evidence for a photosensitizing action. The clinical reaction of a burning erythema in the skin exposed to sunlight is typical of one form of phototoxicity. The two subjects who were demonstrated to be photosensitive by phototesting could not be distinguished from the other subjects taking etretinate by skin type, drug dosage, or duration of treatment. This suggests an idiosyncratic reaction which might be explained in terms of individual variations in the pharmacokinetics or metabolism of the drug. The persistence of a minimal photosensitivity in Subject 9 for one month after stopping etretinate fits well with the long half-life of the drug and its metabolites in the body (Rollman & Valquist, 1983).

We have reported previously that desethylamiodarone, the major metabolite of the antidisrhythmic drug amiodarone, has a greater phototoxic potential than that of the parent drug (Ferguson *et al.*, 1985). Similar observations have been described for chlorpromazine and its demethylated metabolites (Ljunggren & Möller, 1977). The finding that etretinate has no phototoxic potential, while its major metabolite the carboxylic acid, which is similar in structure to all-*trans*-retinoic acid (Fig. 1), is active, appears unique for drug-induced photosensitivity. However, the concentration of Ro 10–1670 required for photohaemolysis is high in relation to reported serum levels (Rollman & Valquist, 1983) and, in general, higher levels are required for phototoxicity *in vivo* than those which produce photohaemolysis.

The lack of unequivocal evidence for photosensitivity due to isotretinoin in our study appears at variance with the findings of a phototoxic potential *in vitro* and with reports from the U.S.A. However, the subjects in our study were on a lower dose regimen than that used in the U.S.A. where there may also be an additional factor of increased levels of sunlight. As with Ro 10–1670, the concentration of isotretinoin required to produce photohaemolysis is high in relation to reported plasma levels in subjects taking the drug. Where photosensitivity due to isotretinoin

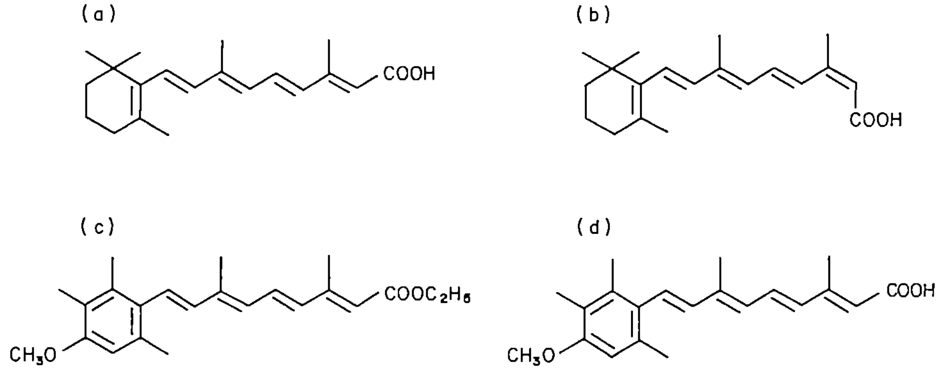

FIGURE 1. Molecular structures of (a) tretinoin (all-*trans*-retinoic acid); (b) isotretinoin (13-*cis*-retinoic acid; Roaccutane); (c) etretinate (Tigason), and (d) etretinate metabolite (Ro 10-1670).

has been reported, it may well be due to individual variation in the pharmacology of the drug since it would seem that given a sufficient dosage of the drug and appropriate radiation levels, reactions should be produced in the skin.

While on clinical and investigative grounds phototoxicity seems a probable explanation for any photosensitivity due to etretinate, an alternative hypothesis, based on the thinning of the stratum corneum and consequent reduction in its optical barrier properties, associated with retinoid therapy, may be proposed. However, according to Claesson, Juhlin and Wettermark (1959) and van der Leun (1966), complete removal of the stratum corneum results in only a 3-fold increase in photosensitivity, whereas we obtained MEDs lowered by an order of magnitude or more. Moreover, the other subjects on retinoid therapy experienced the dryness and increased fragility typical of epidermal adverse effects, which might have been expected to result in increased photosensitivity if this was the mechanism involved.

Photohaemolysis with the retinoids was obtained using UVA wavelengths from 315–400 nm, the peak emission from the lamps being around 355 nm. The wavelength dependency for this effect requires further study, and shorter wavelength UV may well be more effective. Where phototesting was positive in the etretinate treated subjects, a broad spectrum of photosensitivity around 315 nm was evident. Accordingly, the one subject with a clinical photosensitivity associated with etretinate, who has restarted the drug, now uses broad spectrum photoprotection including clothing and a zinc oxide containing sunscreen, and this has produced an apparent reduction in his photosensitivity problem.

In this study, we have been able to confirm that etretinate may induce abnormal cutaneous photosensitivity in some subjects and have established that both isotretinoin and the major metabolite of etretinate have a phototoxic potential which may explain the considerable numbers of anecdotal reports of photosensitivity accompanying the use of the retinoids in dermatological therapy. The concentrations of the retinoids required to demonstrate phototoxicity are relatively high and this may also explain why abnormal photosensitivity does not occur in all subjects taking the drugs.

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# Potentiation of the generation of reactive oxidants by human phagocytes during exposure to benoxaprofen and ultraviolet radiation *in vitro*

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## SUMMARY

The effects of ultraviolet (UV) radiation on the spontaneous membrane-associated oxidative metabolism of human polymorphonuclear leukocytes (PMNL) and mononuclear leukocytes (MNL), co-incubated in the presence and absence of the non-steroidal, anti-inflammatory drug (NSAID) benoxaprofen at various concentrations, were investigated *in vitro*. Assays of superoxide generation and luminol-enhanced chemiluminescence (CL) were used to detect the production of reactive oxidants by PMNL and MNL. Benoxaprofen in the absence of UV radiation caused a dose-related activation of superoxide production and CL by PMNL. Exposure of PMNL to UV radiation in the absence of benoxaprofen caused a slight increase in superoxide generation and an unsustained slight increase in CL within 10 s. Exposure of both MNL and PMNL to UV radiation in the presence of benoxaprofen had a marked synergistic effect on both superoxide generation and CL. These effects were also achieved with UVA irradiation but were not detected when adherent cell depleted MNL or sodium fluoride (NaF) ($10^{-2}$ M) pulsed PMNL, or PMNL from children with chronic granulomatous disease, were used. The pro-oxidative effects of benoxaprofen and UV radiation alone and in combination are dependent on intact phagocyte membrane-associated oxidative metabolism. It is postulated that the pro-oxidative interactions which occur between human phagocytes, benoxaprofen and ultraviolet radiation cause the dermatological side-effects of benoxaprofen.

Photosensitivity is a recognized cutaneous side-effect of many systemic drugs (Levene & Magnus, 1969; Harber & Baer, 1972). These reactions are classified as photoallergic if underlying immunological mechanisms can be demonstrated. In the absence of detectable immunological mechanisms the reactions are termed phototoxic. The molecular basis of phototoxic reactions has not been established. However, there appears to be a requirement for the participation of molecular oxygen, at least in some drug-mediated phototoxic reactions (Ito,

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1978; Joshi & Pathak, 1984). Photodynamic interactions involving the photosensitizing drug, ultraviolet radiation (UVR) and oxygen lead to the generation of reactive oxidants such as singlet oxygen, superoxide, hydroxyl radicals and hydrogen peroxide (Joshi & Pathak, 1984). These reactive oxidants may promote photo-oxidative damage to cell membranes as well as blood vessel dilatation, oedema and erythema responses (Anderson *et al.*, 1974; Joshi & Pathak, 1984).

The non-steroidal, anti-inflammatory drug (NSAID) benoxaprofen has been reported to cause a particularly high incidence of phototoxic reactions (Webley, 1981; Halsey & Cardoe, 1982). Ferguson *et al.* (1982) reported that benoxaprofen had the potential to cause photosensitivity in all subjects. Other reported cutaneous side-effects of benoxaprofen included milia (Dawes, Ramon & Haslock, 1982; Stuart, 1982), onycholysis (Halsey & Cardoe, 1982; Hindson *et al.*, 1982), and eruptive skin tumours (Findlay & Hull, 1982). We have reported previously that benoxaprofen possesses pro-oxidative properties and activates the membrane associated oxidative metabolism of human polymorphonuclear leukocytes (PMNL) and mononuclear leukocytes (MNL) *in vitro* at pharmacological concentrations (Anderson, 1983; Anderson *et al.*, 1984; Eftychis & Anderson, 1984). Our findings provided evidence that the pro-oxidative effects of benoxaprofen were related to the anti-inflammatory and immunosuppressive properties of the drug by promoting auto-oxidative inhibition of PMNL and MNL migration and induction of non-specific suppressor activity in MNL (Anderson *et al.*, 1984; Naudé *et al.*, 1983; Eftychis & Anderson, 1984). Furthermore, we suggested that the cutaneous side-effects of the drug may have been related to potentiation of free radical generation by benoxaprofen-primed phagocytes during exposure to UV radiation. The possible participation of phagocytes, with their potent capacity for transforming molecular oxygen into reactive oxidants, in phototoxicity reactions is a concept which merits further investigation.

We are in agreement with Allen (1983) that there are important lessons to be learned from the benoxaprofen experience. The results presented in this report may be of value in predicting and controlling the cutaneous side-effects of pro-oxidative drugs as well as providing insight into the anti-inflammatory, immunosuppressive mechanisms of a sub-group of NSAID's which are pro-oxidative.

## METHODS

### *Benoxaprofen*

The water-soluble sodium salt of benoxaprofen, 2-(4-chlorophenyl)-α-methyl-5-benzoxazole acetic acid (Lilly Research Centre Ltd, Windlesham, Surrey, U.K.) was used for these investigations at final concentrations of 15, 30 and 60 μg/ml which are well within the therapeutic range (Kamal & Koch, 1982; Naudé & Anderson, 1982).

### *Leukocyte preparation*

PMNL and MNL were separated on Ficoll:metrizoate cushions and residual erythrocytes in the PMNL preparations were removed by sequential sedimentation with 3% gelatin and selective lysis with 0·85% ammonium chloride (Anderson *et al.*, 1984; Eftychis & Anderson, 1984). PMNL and MNL were resuspended in indicator-free Hanks' balanced salt solution (HBSS, Grand Island Biological Co., Paisley, Scotland) pH 7·4.

### *UV irradiation of PMNL and MNL*

The UV source was a new, previously unused Philips MLV irradiation lamp, 300 W (Philips Electronić Ltd, Johannesburg, South Africa) emitting, at 50 cm from the lamp according to manufacturer's data, UVA 1·69 mW/cm$^2$, UVB 0·88 mW/cm$^2$ and UVC 0·01 mW/cm$^2$

respectively. On the basis of preliminary experiments, a 1 min exposure time was chosen which corresponded to 0·13 J/cm$^2$ UVA. To investigate the effects of UVA irradiation, a 2 mm thickness Schott WG4 optical filter which excludes radiation of wavelength less than 320 nm was placed over the polypropylene vials containing the leukocyte suspensions to exclude UVB and UVC. Using a Waldman meter, the UVA emission was 1·90 mW/cm$^2$ at a distance of 50 cm after exclusion of UVB and UVC.

### *Luminol-enhanced chemiluminescence of PMNL and MNL*

$10^5$ PMNL or $10^6$ MNL were incubated in polypropylene vials (45 mm × 10 mm) with $10^{-4}$ M luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma Chemical Co., St Louis, MO, U.S.A.) in the presence and absence of benoxaprofen at concentrations of 15, 30 and 60 $\mu$g/ml in a final volume of 200 $\mu$l HBSS, and exposed to unfiltered and filtered UV light as described above. Identical, non-UV exposed, drug-treated and control systems were included. PMNL and MNL were pre-incubated at 37°C for 10 min, benoxaprofen was added and the preparations irradiated immediately. After irradiation, the PMNL and MNL were re-incubated at 37°C and luminol enhanced chemiluminescence was measured in a Lumac Biocounter (Model 2010, Lumac Systems Inc., Titusville, Florida, U.S.A.) after 1, 3, 5, 10 and 15 min of incubation. The results are expressed as relative light units (r.l.u.)/10 s.

PMNL and MNL-free control systems were included to monitor the following: (a) spontaneous chemiluminescence of luminol ± UV light, (b) luminol and benoxaprofen ± UV light, and (c) HBSS ± UV light.

### *Superoxide generation by PMNL*

PMNL ($5 \times 10^6$/ml) were incubated in plastic Petri dishes (55 mm diameter, Nunclon®, Kamstrup, Denmark) with 0·1 mM ferricytochrome c (cyt c type VI, Sigma Chemical Co.) with and without 200 units/ml of superoxide dismutase (Sigma Chemical Co.). The dishes, which contained a final volume of 3 ml HBSS, were pre-incubated for 10 min at 37°C prior to the addition of benoxaprofen and UV irradiated for 1 min. After irradiation, the dishes, as well as non-irradiated control systems with and without benoxaprofen, were re-incubated at 37°C and 0·5 ml aliquots removed after 1, 3, 5, and 10 min of incubation. The aliquots were transferred immediately to test tubes containing 2·5 ml of ice cold 0·15 M phosphate-buffered saline (PBS, pH 7·2) centrifuged in a refrigerated centrifuge at 4°C to remove the PMNL and the supernatants assayed for reduced cyt c spectrophotometrically at 550 nm. The amount of reduced cyt c was calculated using an absorbance coefficient of 15·5 mM at 550 nm (Margoliash & Frohwirt, 1959). Superoxide-dependent reduction of cyt c was expressed as the difference in cyt c reduction between reaction mixtures containing no superoxide dismutase and those containing 200 units/ml superoxidase dismutase. These results are expressed as n moles superoxide production/$5 \times 10^6$ PMNL.

In PMNL-free control systems, cyt c was irradiated in HBSS ± superoxide dismutase in the presence and absence of benoxaprofen.

### *Studies with NaF-pulsed PMNL from normal individuals and PMNL from patients with chronic granulomatous disease*

To investigate the requirement for intact cellular oxidative metabolism NaF-pulsed normal PMNL and PMNL from three children with the autosomal recessive form of chronic granulomatous disease (CGD) were used. NaF pulsing of PMNL causes irreversible exhaustion of membrane-associated oxidative metabolism (Matzner *et al.*, 1982), whilst there is a congenital absence of superoxide generating mechanisms in CGD leukocytes. Normal PMNL were incubated with $10^{-2}$ M NaF for 40 min, washed, and resuspended in HBSS. Assays of luminol-enhanced CL to investigate the effects of benoxaprofen with and without UV light exposure were performed as above using NaF-pulsed and CGD PMNL.

### *Removal of adherent cells from MNL*

MNL were depleted of adherent cells by a one-step incubation of MNL ($5 \times 10^6$/ml) on plastic Petri dishes (Falcon, Oxnard, California, U.S.A.) for 60 min at 37°C. The non-adherent cells were removed and residual monocytes enumerated using a cytochemical method for the detection of non-specific esterase activity (Li, Lam & Lam, 1973). The effects of benoxaprofen and/or UV irradiation on the luminol-enhanced CL of MNL were compared with the corresponding responses in adherent cell depleted MNL.

### *Effects of UV irradiation on the pro-oxidative activity of benoxaprofen*

In these experiments, benoxaprofen (15 μg, 30 μg and 60 μg/ml HBSS), in the absence of PMNL, was irradiated as described above for 1 min and 5 min. The effects of irradiated and non-irradiated control preparations of the drug on luminol-enhanced CL of PMNL were then compared.

### *Expression and statistical analyses of results*

The results are expressed as the mean and standard error for each series of experiments. Statistical analyses of results were performed using paired *t*-tests to compare the results obtained in each experiment with those from a corresponding control, using cells obtained from the same subject on the same day and tested at the same time.

## RESULTS

### *Effects of benoxaprofen and/or UV irradiation on luminol-enhanced chemiluminescence of PMNL and MNL*

Co-incubation of PMNL with benoxaprofen caused a statistically significant increase in the spontaneous membrane-associated oxidative metabolism measured by CL ($P < 0{\cdot}025$–$P < 0{\cdot}005$). Exposure of PMNL to UV light caused an immediate (within 10 s), unsustained increase in CL. Exposure of PMNL in the presence of benoxaprofen to UV light caused a marked enhancement of luminol enhanced CL which was significantly greater than the corresponding results obtained with PMNL and benoxaprofen only. These interactive effects of benoxaprofen and UV light were synergistic at 1, 3 and 5 min of incubation at 37°C, and thereafter additive. The results of kinetic studies with 60 μg/ml benoxaprofen are shown in Figure 1 and the results obtained with all three concentrations of the drug after 1, 3 and 5 min are shown in Table 1. Similar results were obtained with MNL and these are shown in Table 2.

No detectable CL above background levels was observed in cell-free control systems, i.e. for irradiated luminol only or for irradiated benoxaprofen with and without luminol.

### *Effects of benoxaprofen and/or UV irradiation on superoxide generation by PMNL*

Co-incubation of PMNL with benoxaprofen only was associated with increased superoxide generation. Exposure of PMNL, in the absence of benoxaprofen, to UV light also increased superoxide generation, although modestly relative to benoxaprofen. However, UV exposure of PMNL co-incubated with benoxaprofen caused a striking enhancement of superoxide generation by PMNL which was synergistic after 1, 3 and 5 min of incubation at 37°C. Results

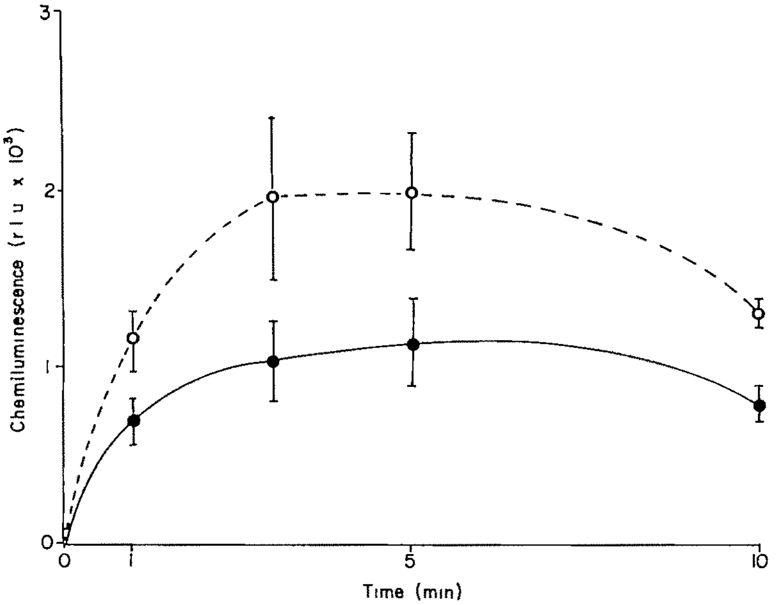

FIGURE 1. The effects of incubation with benoxaprofen (30 μg/ml) only (●——●), or benoxaprofen and UV irradiation (○- - - -○), on PMNL luminol-enhanced chemiluminescence. Points are means ± SEM. Background values are shown in Table 1.

TABLE 1. The effects of benoxaprofen and ultraviolet light individually and in combination on PMNL superoxide production and spontaneous chemiluminescence

| | nmol superoxide per $5 \times 10^6$ PMNL* | | | PMNL chemiluminescence** | | |
|---|---|---|---|---|---|---|
| Test system | 1 min | 3 min | 5 min | 1 min | 3 min | 5 min |
| No treatment | 1·6 ± 0·2 | 8·2 ± 2·2 | 14 5 ± 1·0 | 387 ± 108 | 501 ± 144 | 649 ± 232 |
| UV-exposure | 3·4 ± 0·2 | 15·4 ± 1·1 | 17·3 + 1·0 | 474 ± 142‡ | 512 ± 148 | 614 ± 185 |
| Benoxaprofen 15 μg/ml | ND | ND | ND | 608 ± 135 | 892 ± 286 | 968 ± 345 |
| UV-exposure + benoxaprofen 15 μg/ml | ND | ND | ND | 783 ± 239† | 1491 ± 641† | 1516 ± 588† |
| Benoxaprofen 30 μg/ml | 4·3 ± 0 2 | 15·8 ± 0·5 | 19·4 ± 1·3 | 736 ± 102 | 1031 ± 209 | 1166 ± 231 |
| UV-exposure + benoxaprofen 30 μg/ml | 10·4 ± 1·0† | 24 4 ± 1·0† | 29 4 ± 1·0† | 1169 ± 163† | 1972 ± 569† | 2000 ± 500† |
| Benoxaprofen 60 μg/ml | 9·6 ± 2 2 | 16·2 ± 0·4 | 24·0 ± 1·0 | 912 ± 153 | 2005 ± 689 | 2039 ± 763 |
| UV-exposure + benoxaprofen 60 μg/ml | 17·4 ± 1·8 | 27·0 ± 1 0† | 36·0 ± 1 3† | 1404 ± 238† | 2946 ± 927† | 2508 ± 852† |

* Results are the mean ± SEM of six different experiments.

** Results expressed in relative light units/10 s/$10^5$ PMNL. Values are mean ± SEM of six different experiments.

† $P < 0 025$–$P < 0{\cdot}005$; ‡ $P < 0{\cdot}05$: for comparison of UV + benoxaprofen systems with benoxaprofen only.

ND = Not done.

TABLE 2. The effects of benoxaprofen and UV irradiation individually and in combination on luminol-enhanced chemiluminescence of MNL

| Test system | MNL chemiluminescence* | | |
|---|---|---|---|
| | 1 min | 3 min | 5 min |
| No treatment | 177 ± 80* | 526 ± 212 | 564 ± 146 |
| UV irradiation | 253 ± 104 | 494 ± 155 | 514 ± 119 |
| Benoxaprofen 30 μg/ml | 880 ± 320 | 1333 ± 456 | 1394 ± 540 |
| Benoxaprofen 30 μg/ml + UV irradiation | 1213 ± 371† | 1811 ± 610† | 1770 ± 617† |
| Benoxaprofen 60 μg/ml | 469 ± 179 | 988 ± 388 | 1460 ± 788 |
| Benoxaprofen 60 μg/ml + UV irradiation | 1472 ± 766† | 1961 ± 825† | 1975 ± 732† |

* Results expressed in relative light units/10s/$10^6$MNL. Values are means ± SEM of six different experiments.

† $P<0.05$–$P<0.005$: for comparison of UV-exposed systems with the corresponding control systems.

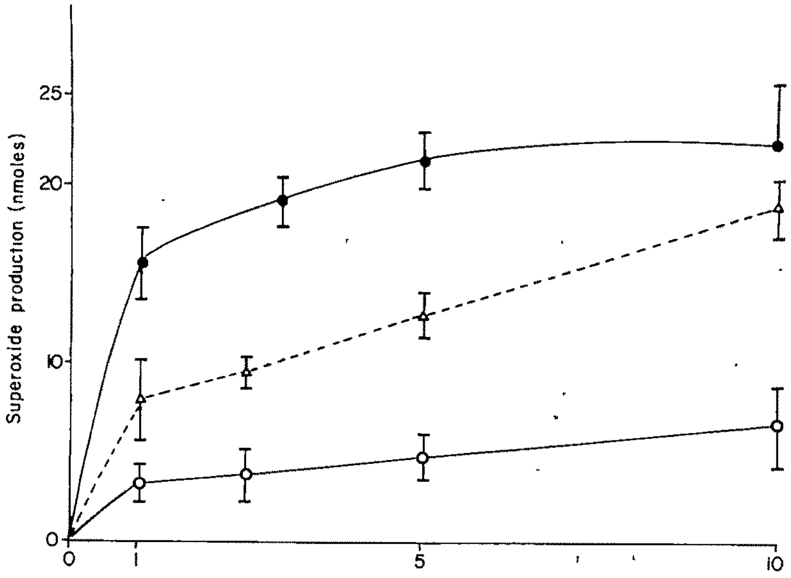

FIGURE 2. The effects of exposure to UV radiation only (○——○), or 60 μg/ml benoxaprofen only (△- - - -△), or UV radiation in the presence of benoxaprofen (●——●), on superoxide generation by PMNL. Points are means ± SEM.

of kinetic studies with 60 μg/ml of benoxaprofen are shown in Figure 2 and results obtained with all three concentrations of the drug are shown in Table 1.

Irradiation of cyt c with or without superoxide dismutase in the presence (15, 30 and 60 μg/ml) and absence of benoxaprofen did not result in detectable superoxide generation.

TABLE 3. The effects of UV and UVA irradiation on PMNL and MNL luminol-enhanced chemiluminescence in the presence and absence of benoxaprofen (30 μg/ml)

| Test system | Luminol-enhanced chemiluminescence* MNL | PMNL |
|---|---|---|
| Untreated | 288 ± 51 | 410 ± 41 |
| UV irradiation | 278 ± 55 | 406 ± 121 |
| UVA irradiation | 307 ± 60 | 421 ± 116 |
| Benoxaprofen 30 μg/ml | 1367 ± 420 | 913 ± 312 |
| Benoxaprofen 30 μg/ml and UV irradiation | 1610 ± 520 | 1714 ± 410 |
| Benoxaprofen 30 μg/ml and UVA irradiation | 1941 ± 720 | 1912 ± 486 |

* Results expressed in relative light units/10 s/$10^6$ MNL or $10^5$ PMNL. Values are mean ± SEM of three different experiments using 5 min incubation.

*Effects of benoxaprofen and/or UVA irradiation on luminol-enhanced chemiluminescence of PMNL and MNL*

Essentially similar results to those described above were obtained with UVA. The results obtained after 5 min of incubation at 37°C for PMNL and MNL exposed to UVA radiation in the presence of 30 μg/ml benoxaprofen are shown in Table 3.

*Investigations using NaF-pulsed and CGD PMNL*

NaF pulsing completely abolished the membrane-associated oxidative metabolism of PMNL. For untreated PMNL the resting and benoxaprofen (30 μg/ml) activated CL results were 581 ± 121 and 1201 ± 247 relative light units (r.l.u.) after 5 min of incubation. The corresponding values with NaF-pulsed PMNL were 19 ± 1 and 20 ± 1 r.l.u. (the background value for medium and luminol was 9 ± 0·5). Likewise, CGD PMNL were unresponsive to the pro-oxidative effects of benoxaprofen and/or UV irradiation. The values for untreated, UV-treated, benoxaprofen-treated and UV and benoxaprofen-treated CGD PMNL were 12 ± 1, 17 ± 1, 15 ± 3 and 23 ± 3 r.l.u. respectively.

*Investigations using adherent cell depleted MNL*

Removal of adherent cells from MNL populations was associated with the loss of the increased CL response induced by benoxaprofen and/or UV radiation. The values for untreated, UV-treated, benoxaprofen (30 μg/ml)-treated and UV and benoxaprofen-treated MNL were 173, 220, 264 and 441 r.l.u. respectively. The corresponding values for adherent cell-depleted MNL were 15, 24, 16 and 30 r.l.u. respectively (mean values of a single experiment).

*Effects of UV irradiation on the pro-oxidative properties of benoxaprofen*

Benoxaprofen was irradiated for 1 min and 5 min in the absence of PMNL. Control (non-irradiated) or irradiated drugs were then added to PMNL and luminol-enhanced CL measured after 1, 3, 5, 10 and 15 min. After 5 min of incubation, the results were 601 ± 143, 1316 ± 312 and 1381 ± 235 r.l.u. for PMNL only, PMNL + 30 μg/ml benoxaprofen and PMNL + 30 μg/ml

TABLE 4. Measurement of the effects of addition of benoxaprofen before and after UV irradiation on PMNL luminol-enhanced chemiluminescence

| Test system | Luminol-enhanced chemiluminescence* |
|---|---|
| Untreated control | 376 ± 101 |
| UV irradiation only | 424 ± 123 |
| Benoxaprofen 60 μg/ml | 920 ± 256 |
| Benoxaprofen 60 μg/ml added before UV irradiation | 1928 ± 381 |
| Benoxaprofen 60 μg/ml added after UV irradiation | 785 ± 201 |

* Results expressed in relative light units/10 s/$10^5$ PMNL. Values are mean ± SEM of three different experiments using 5 min incubation.

irradiated benoxaprofen respectively (mean value ± SEM of three experiments). UV irradiation of benoxaprofen did not alter the pro-oxidative properties of the drug.

### *Effects of exposure of PMNL to UV radiation with subsequent addition of benoxaprofen on luminol-enhanced chemiluminescence*

In these experiments benoxaprofen (60 μg/ml) was added to PMNL after 1 min of UV irradiation and luminol-enhanced CL compared with systems in which the drug was present during irradiation of PMNL. These results (Table 3) show a requirement for the presence of benoxaprofen during UV irradiation of PMNL to achieve the synergistic enhancing effect of the drug and UV on PMNL CL. The synergistic effect was not detectable when benoxaprofen was added after irradiation of PMNL. These results are shown in Table 4.

### *Viability studies*

Benoxaprofen only (all concentrations), UV irradiation only or UV irradiation in the presence of benoxaprofen did not affect PMNL viability which remained at >90% using the eosin (0·1%) exclusion method.

## DISCUSSION

Despite reports of the beneficial therapeutic effects of benoxaprofen in rheumatoid arthiritis and inflammatory diseases of the skin (reviewed by Allen, 1983), the drug was withdrawn from the international market in 1982 because of the high incidence of side-effects. Novel mechanisms of anti-inflammatory activity such as preferential inhibition of 5-lipoxygenase and selective inhibition of the migration of MNL were attributed to benoxaprofen (Walker & Dawson, 1979; Meacock & Kitchen, 1979). The clinical significance of the inhibitory effects of the drug on leukotriene production was not established (Salmon *et al.*, 1984) and others reported that PMNL and MNL were equally sensitive to the migration-inhibitory effects of benoxaprofen (Naudé *et al.*, 1983; Anderson *et al.*, 1984). We observed that benoxaprofen, unlike a series of other commonly-used NSAIDs, possessed pro-oxidative properties and activated the membrane-associated oxidative metabolism of both PMNL and MNL (Anderson *et al.*, 1984; Eftychis & Anderson, 1984). By increasing the generation and release of auto-reactive oxidants, the drug promoted the auto-oxidative inhibition of PMNL and MNL migration and induction of non-specific suppressor activity in MNL (Anderson *et al.*, 1984; Eftychis & Anderson, 1984).

We proposed a direct relationship between the pro-oxidative properties of benoxaprofen and drug-mediated, anti-inflammatory, immunosuppressive mechanisms. This represents a radical departure from classical concepts of regulation of inflammation by NSAIDs. We also suggested that the cutaneous side-effects of the drug were linked to the pro-oxidative, immunosuppressive mechanisms.

Activated phagocytes transform molecular oxygen to stable and unstable biologically reactive oxidants which are mutagenic (Weitzman *et al.*, 1985), and immunosuppressive (Zoschke & Messner, 1984) and which may cause phototoxicity reactions (Joshi & Pathak, 1984). It seemed possible that benoxaprofen, in addition to spontaneous activation of membrane associated oxidative metabolism in human phagocytes, primes these cells to UV radiation leading to even greater production of reactive oxidants and development of cutaneous side-effects such as phototoxicity reactions and eruptive skin tumours.

The synergistic interaction of benoxaprofen and UV radiation reported in this present investigation was dependent on intact phagocyte membrane-associated oxidative metabolism. No evidence was obtained to support either the generation of a photo-activated form of benoxaprofen with increased pro-oxidative activity or a reactive oxidant such as superoxide or $H_2O_2$ as a consequence of benoxaprofen-UV interactions. We confirmed the observation of Ferguson *et al.* (1982) that the active component of the UV spectrum was UVA.

The molecular/pharmacological basis of the pro-oxidative properties of benoxaprofen has not yet been elucidated. It is possible that the drug is a partial agonist of leukoattractant receptors on phagocytes, causing selective activation of membrane-associated oxidative metabolism, but not chemotaxis. Alternatively, benoxaprofen may affect oxidant generation by activating the enzyme protein kinase C in PMNL and MNL (Castagna *et al.*, 1982). Further investigations are required to establish the molecular mechanisms of the synergistic effects of benoxaprofen and UV radiation on the activation of membrane-associated oxidative metabolism in human phagocytes.

Since our investigations were performed with blood PMNL and MNL, the relevance of our findings to benoxaprofen-induced cutaneous side-effects may be questioned. However, it is reasonable to assume that Langerhans cells in the skin share common mechanisms of generation of reactive oxidants with PMNL and MNL. Langerhans cells may be activated by benoxaprofen and UV radiation as described here for PMNL and MNL.

Cutaneous side-effects may restrict future development of pro-oxidative NSAIDs such as benoxaprofen, and, while phototoxicity reactions may be acceptable in some cases, the potential mutagenic activity of phagocyte-derived oxygen radicals is more ominous (Weitzman *et al.*, 1985). However, it may be possible to improve the safety of pro-oxidative drugs designed for chemotherapeutic and/or photochemotherapeutic clinical applications by subjecting them to the type of laboratory investigations described in this report. For instance, significant pro-oxidative activity, with and without UV irradiation, was achieved with 15 $\mu$g/ml benoxaprofen. Serum levels of over 250 $\mu$g/ml were found in some female subjects during ingestion of 600 mg of benoxaprofen daily (Naudé & Anderson, 1982). It may be possible to determine the dose of a pro-oxidative agent such that anti-inflammatory and immunosuppressive effects are retained and cutaneous side-effects minimized.

We conclude that there exists a sub-class of NSAIDs, of which benoxaprofen is the prototype, which mediate anti-inflammatory and immunosuppressive activities as well as cutaneous side-effects by UV potentiated, pro-oxidative interactions with human phagocytes.

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# Ionophore A23187 stimulates arachidonic acid release and cyclic GMP accumulation in pig epidermis

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## SUMMARY

We studied the effect of Ionophore A23187 (A23187) on phospholipid degradation and the accumulation of cyclic GMP in pig epidermis. A23187 stimulated the release of AA, probably partially through the activation of phospholipase $A_2$. A23187 also stimulated the accumulation of epidermal cyclic GMP, but the activity of cyclic GMP-dependent phosphodiesterases was not altered. Mepacrine, an inhibitor of phospholipase $A_2$, inhibited the A23187-stimulated accumulation of cyclic GMP. The results suggest that A23187 stimulates the accumulation of epidermal cyclic GMP by a calcium-dependent process which also requires products of phospholipid degradation.

Ionophore A23187 (A23187) is of considerable experimental and theoretical interest, because it specifically carries divalent cations, especially calcium, across cell membranes without disturbing the $Na^+$-$K^+$ balance, i.e. the membrane potential (Pressman, 1976; Reed & Lardy, 1972). It has been used for inducing many calcium-dependent physiological reactions such as secretion (Scheele & Haymovits, 1980), platelet aggregation (Nishikawa, Tanaka & Hidaka, 1980), and cell proliferation (Marks *et al.*, 1981). In mouse epidermis, the topical application of A23187 can induce inflammatory reactions and hyperproliferative changes (Marks *et al.*, 1981).

It has been postulated that most tissues possess two major classes of receptors for controlling cellular functions and proliferation. One class triggers the production of cyclic AMP, while the other induces inositol phospholipid turnover, calcium mobilization, and frequently arachidonic acid (AA) release and cyclic GMP production (Nishizuka, 1984). Ohsako and Deguchi (1981) also reported that phosphatidic acid, as a calcium ionophore, stimulated the translocation of

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calcium across cell membranes and, as a consequence, induced the synthesis of cyclic GMP in neuroblastoma cells.

One of the important actions of A23187 is the stimulation of phospholipases, the release of AA and the production of AA metabolites.

We report here on the effect of A23187 on the release of AA and the synthesis of cyclic GMP in pig epidermis.

## METHODS

Chemicals and drugs were obtained from the following sources: calcium-free RPMI 1640, RPMI 1640 (Microbiological Associates, Walkersville, MD, U.S.A.), Ionophore A23187 (Calbiochem-Behringer Corp., La Jolla, CA, U.S.A.), hydrocortisone, bovine serum albumin (BSA), mepacrine, HEPES, cyclic GMP (Sigma Chemical Co., St Louis, MO, U.S.A.), phosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidyl-ethanolamine (PL Biochemicals, Milwaukee, Wisconsin, U.S.A.), Dispase (Godo Shusei, Tokyo, Japan), 1-$^{14}$C-arachidonic acid (specific activity 58 mCi/mmol) (Amersham Japan, Tokyo, Japan). All other chemicals and drugs were of analytical reagent grade.

Skin slices were taken from the backs of 6–8 kg domestic pigs anesthetized with Nembutal (20–30 mg/kg i.p.) using a Castroviejo keratome set at a depth of 0·2 mm. The skin slices obtained contained 80–90% epidermis (Yoshikawa *et al.*, 1975), and are referred to as 'epidermal slices'. The epidermal slices were kept in RPMI 1640 medium at 4°C, cut into 5 × 10 mm pieces and used within 30 min. To obtain pure epidermis, epidermal slices (0·3 mm in thickness) were treated with Dispase (1000 IU/ml) at 4°C for 20 h, and the epidermis was peeled off gently from the dermis using forceps. Epidermis obtained by this method is referred to as 'pure epidermal sheets' to distinguish it from the epidermal slices which contain dermis.

Incubations, pre-labelling, and other procedures were carried out as described previously (Aoyagi *et al.*, 1985). Briefly, epidermal slices or pure epidermal sheets, pre-labelled with 0·1 $\mu$Ci/ml 1-$^{14}$C-arachidonic acid ($^{14}$C-AA) for 4 h were washed twice in calcium-free RPMI 1640 medium. They were then treated with A23187 or other compounds for the periods indicated. Two pieces, 5 × 10 mm, of epidermal slices or pure epidermal sheets were used for each assay. The amount of $^{14}$C-radioactivity in the medium was determined by scintillation counting. The radioactivity of $^{14}$C-AA released into the medium represented more than 85% of the total radioactivity recovered.

Pure epidermal sheets were used for the extraction and separation of phospholipids. Samples, 5 × 10 mm, pre-labelled with $^{14}$C-AA for 4 h were treated with A23187 (10 $\mu$M) for 10 min. Samples were removed at 0 and 10 min, and frozen immediately between two dry-ice plates. After extraction of phospholipids, these were spotted on Silica Gel G plates and the plates were developed with chloroform:methanol:28% aqueous ammonia (60:35:8) for the separation of the different phospholipids. Procedures for the analysis of phospholipids were the same as described previously (Aoyagi *et al.*, 1985).

Epidermal slices were cut into 7 × 7 mm pieces and then pre-incubated in modified Krebs-Ringer buffer (KRB) with HEPES (20 mM, pH 7·4) at 37°C for 15 min to standardize the cyclic GMP level. After pre-incubation, eight pieces were selected at random and floated with the keratin layer uppermost in KRB with HEPES containing various chemicals and drugs. Isobutylmethylxanthine (IBMX), an inhibitor of phosphodiesterase, which has been reported to potentiate cyclic GMP levels significantly in this system (Iizuka *et al.*, 1979a), was added to the incubation medium in some experiments. After incubation, the samples were frozen immediately between two dry-ice plates and kept at −70°C until use. The procedure for extraction of cyclic GMP was the same as previously described (Iizuka *et al.*, 1979a). Briefly, cyclic nucleotides were extracted by homogenization of the tissue in 1% perchloric acid at 4°C and after removing the perchloric acid by precipitation with 6M KOH, extracts were applied to Dowex-1 columns, 0·7 × 4 cm (chloride form, 1 × 8, 400 mesh) which were pre-washed with formic acid. Cyclic GMP was eluted with 5M formic acid. Cyclic GMP was measured by radioimmunoassay using the Yamasa cyclic GMP kit after one step of succinylation. The incubation medium was lyophilized and reconstituted with distilled water, and cyclic GMP in the medium was also measured by radioimmunoassay.

The activity of cyclic GMP-dependent phosphodiesterase (PDE) was determined by the method of Adachi *et al.* (1976). Enzyme activity was measured after development of thin layer chromatography plates with cellulose in 95% ethanol:1 M ammonium acetate (70:35). The concentration of cyclic GMP used as substrate was 100 μM for the high Km and 1·0 μM for the low Km PDE.

The protein content of the samples was determined by the method of Lowry *et al.* (1951) with BSA as a standard.

## RESULTS

Epidermal slices or pure epidermal sheets pre-labelled with $^{14}$C-AA were treated with 10 μM A23187 for up to 30 min in the presence of calcium (1·2 mM). The results are shown in Figure 1. A23187 stimulated the release of AA as early as 5 min both in epidermal slices and in pure epidermal sheets. During a 30 min incubation, the stimulatory effect of A23187 did not appear

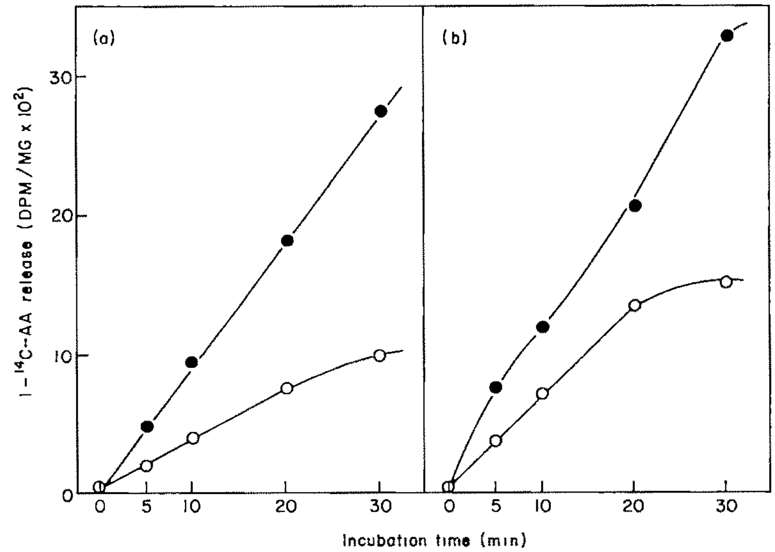

FIGURE 1. Effect of A23187 on the release of AA from epidermal slices (a) and pure epidermal sheets (b) pre-labelled with $^{14}$C-AA (0·1 μCi/ml). ●—● with A23187 (10 μM); ○—○ control. Points are means of duplicate determinations.

TABLE 1. Effect of calcium on A23187-stimulated AA release from epidermal slices

| | AA release (dpm/tube) | |
|---|---|---|
| A23187 | $Ca^{2+}$ 0 mM | $Ca^{2+}$ 1·2 mM |
| 0 | 1475 (1458–1492)** | 1489 (1509–1469) |
| 1 μM | 1625 (1632–1618) | 1643 (1649–1636) |
| 5 μM | 1762 (1767–1756) | 1913 (1917–1908) |
| 10 μM | 1885 (1850–1919) | 2718 (2545–2890) |

* EGTA 0·1 mM was added to the medium without calcium.

** Values are means and ranges of duplicate determinations.

TABLE 2. Effect of mepacrine on A23187-stimulated AA release from epidermal slices in the presence of 1·2 mM calcium

| | AA release (dpm/tube) | |
|---|---|---|
| Mepacrine | A23187 0 μM ($n=2$) | A23187 10 μM ($n=3$) |
| 0 | 2834 (5) | 4926 (±154) |
| 0·01 μM | 2914 (113) | 5047 (±214) |
| 0·1 μM | 2394 (36) | 4605 (±263) |
| 1 μM | 2885 (436) | 4044 (±168) |
| 10 μM | 2404 (70) | 3855 (±134) |

Values are means of duplicate or triplicate determinations. Values in parentheses are differences between duplicate readings where $n=2$, and ± SEM where $n=3$.

TABLE 3. Degradation of $^{14}C$-labelled phospholipids in pure epidermal sheets by A23187

| | Phospholipids (dpm/tube) | | |
|---|---|---|---|
| Time (min) | PI + PS | PC | PE |
| 0 ($n=2$) | 8140 (944) | 8471 (476) | 6283 (4) |
| 10 ($n=3$) | 4380 (±110) | 4712 (±262) | 3854 (±363) |

Values are means of duplicate or triplicate determinations. Values in parentheses are differences between duplicate readings where $n=2$, and ± SEM where $n=3$.

TABLE 4. Effect of A23187 on intracellular and extracellular cyclic GMP accumulation in epidermal slices

| | | Cyclic GMP fmol/mg protein<br>Ca$^{2+}$ absent | Ca$^{2+}$ (1 mM) |
|---|---|---|---|
| No addition | I | 15·9±2·56 | 20·7±3·30 |
| | E | 14·8±1·13 | 29·0±3·10 |
| A23187 (5 μM) | I | 16·2±2·40 | 30·6±4·70* |
| | E | 13·6±1·16 | 31·3±2·82 |
| A23187 (10 μM) | I | 15·4±2·44 | 29·3±4·02** |
| | E | 13·0±1·05 | 31·9±2·76 |

Values are means ± SEM of duplicate determinations of intracellular cyclic GMP (I) from five separate experiments, and extracellular cyclic GMP (E) from six separate experiments.

* Significantly different from control level (with calcium, without A23187) $P < 0·025$; ** $P < 0·05$ (Student's *t*-test).

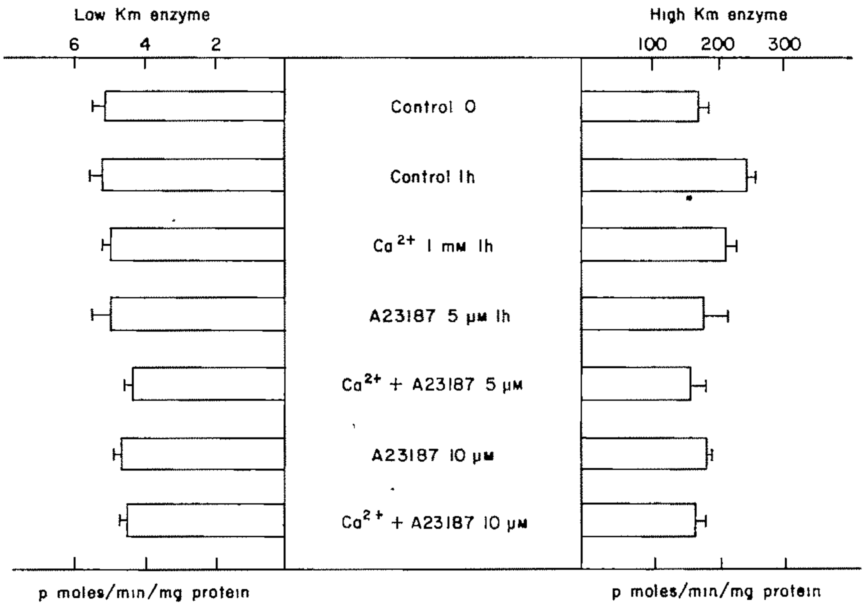

FIGURE 2. Effect of A23187 on cyclic GMP-dependent phosphodiesterase activity in epidermal slices incubated with or without A23187 in the presence or absence or calcium (1·0 mM) at 37°C for 1 h. Bars represent means ± SEM of six determinations.

to have reached its maximum. As shown in Table 1, A23187 alone had only a small effect on the release of AA, while the presence of exogenous calcium greatly potentiated the effect.

The A23187 stimulated release of AA in the presence of calcium 1·2 mM was inhibited by mepacrine, an inhibitor of phospholipase $A_2$, as shown in Table 2. After subtraction of the basal level (in the absence of A23187 and mepacrine), the results showed that 10 μM mepacrine

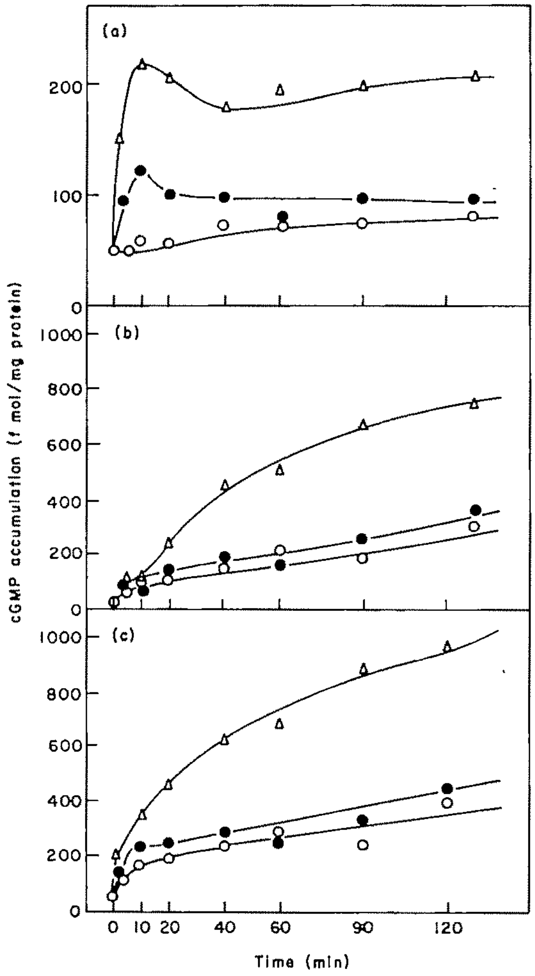

FIGURE 3. Effect of A23187 on cyclic GMP accumulation in epidermal slices incubated in the presence of IBMX (100 $\mu$M). (a) Intracellular; (b) extracellular; (c) total. ● $Ca^{2+}$ 1 mM; △ A23187 10 $\mu$M plus $Ca^{2+}$ 1 mM; ○ control (no A23187 or calcium). Points are means of duplicate determinations.

inhibited A23187-stimulated AA release by 50%, and unstimulated AA release by about 15%.

To detect the source of $^{14}$C-AA released into the medium, $^{14}$C-labelled phospholipids were extracted from pure epidermal sheets and analysed by thin layer chromatography. As shown in Table 3, $^{14}$C-labelled phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidyinositol plus phosphatidylserine (PI+PS), were degraded by A23187.

Epidermal slices were incubated with or without A23187 in the presence or absence of calcium (1 mM) for 1 h, and the levels of intra- and extracellular cyclic GMP were determined. As shown in Table 4, A23187 stimulated significantly the accumulation of intracellular cyclic GMP in the presence of calcium, while it had no effect on the accumulation of cyclic GMP in the absence of calcium. Extracellular cyclic GMP was not affected by A23187 alone, but it was increased in the presence of calcium both with and without A23187.

TABLE 5. Effect of mepacrine on A23187-stimulated cyclic GMP accumulation in epidermal slices

| | Intracellular cyclic GMP fmol/mg protein |
|---|---|
| $Ca^{2+}$ | 204 ± 14 |
| $Ca^{2+}$ + A23187 (10 μM) | 610 ± 116 |
| $Ca^{2+}$ + A23187 (10 μM) + mepacrine (50 μM) | 271 ± 32 |
| $Ca^{2+}$ + A23187 (10 μM) + mepacrine (100 μM) | 279 ± 58 |

Values are mean ± SEM of four determintations.

The activities of both high and low Km phosphodiesterase (PDE) were not significantly altered by treatment with A23187 (Fig. 2).

The time course of A23187-stimulated cyclic GMP accumulation in the presence of IBMX is shown in Figure 3. The level of intracellular cyclic GMP by A23187 stimulation in the presence of calcium reached a maximum after 10 min, was slightly decreased at 40 min, and returned to the maximum between 60 and 120 min incubation.

The effect of mepacrine, an inhibitor of phospholipase $A_2$, on the accumulation of intracellular cyclic GMP stimulated by A23187 in the presence of IBMX, is shown in Table 5. 50 and 100 μM mepacrine inhibited A23187-stimulated accumulation of intracellular cyclic GMP by about 80%. Mepacrine alone had no effect on the level of intracellular cyclic GMP (data not shown).

## DISCUSSION

A23187 is one of the chemicals which stimulate the release of AA in various cell types, probably through calcium influx and subsequent activation of phospholipase $A_2$. In the pig epidermal system, A23187 stimulated significantly the release of AA. This stimulation was inhibited partially by mepacrine. The stimulatory effect on the release of AA and its inhibition by mepacrine has been reported also with epidermal growth factor (EGF) and EGF and A23187 have been found to potentiate the release of AA additively (Aoyagi *et al.*, 1985). As mepacrine partially inhibited A23187-stimulated release of AA, we suggest that A23187 stimulates not only phospholipase $A_2$ activity but also the activity of other phospholipases. The study of phospholipid degradation revealed that A23187 stimulated the release of AA from all the phospholipids tested.

A23187 also stimulated the accumulation of cyclic GMP. There are several endogenous factors which increase epidermal cyclic GMP by activating epidermal guanylate cyclase, including AA, HETE (Cantieri, Graff & Goldberg, 1980), histamine ($H_1$) (Iizuka *et al.*, 1979b), and epidermal growth factor (Aoyagi *et al.*, 1980). However, the mechanism by which cyclic GMP is increased, as well as the role of this cyclic nucleotide, is still not clearly understood. There are several ways in which the accumulation of cyclic GMP can be increased through calcium-dependent processes, including phospholipid degradation, calcium influx and mobilization, AA release and the production of AA metabolites. Although it is not possible to pinpoint the precise process resulting in the accumulation of epidermal cyclic GMP in our studies, we observed that the time course of A23187-stimulated accumulation of cyclic GMP was different from that of release of AA. A23187 stimulated continuously the release of AA during a 30 min incubation period, and the stimulatory effect of A23187 did not reach its maximum within this time, while the accumulation of cyclic GMP stimulated by A23187 reached a maximum level at 10 min, slightly decreased at 40 min, and was maintained at the higher level from 60 to 120 min. We believe that the early accumulation of cyclic GMP could be due to calcium influx, while the later increase in cyclic GMP was probably due to increased levels of AA and of AA metabolites, which have been reported to stimulate epidermal guanylate cyclase (Cantieri, Graff & Goldberg, 1980).

The overall results suggest that phospholipid degradation by a calcium-dependent process induced by A23187 stimulates the accumulation of epidermal cyclic GMP.

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# Immunopathological and ultrastructural findings in human allergic and irritant contact dermatitis

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## SUMMARY

The histopathological features of allergic contact dermatitis were compared with those of irritant contact dermatitis in a group of 17 subjects. Each patient received simultaneous patch tests of a known allergen and a standardized irritant (benzalkonium chloride). The cellular changes occurring between 3 h and 7 days after patch test application were studied by light and electron microscopy and immunocytochemistry.

No differences were observed between the induced allergic contact dermatitis (ACD) and the irritant contact dermatitis (ICD), either in the responding cell types or the sequence of cellular events. Both reactions showed a predominantly T lymphocyte infiltrate with no polymorphonuclear leukocyte involvement. Apposition of Langerhans cells to lymphocytes in the epidermis was seen in both types of response.

Considerable variability in the intensity of reaction to irritant and allergen occurred within individuals. There was no statistically significant difference between the intensity of the reactions to the irritant and the allergen.

There is currently no established means to distinguish allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD). When clinical diagnosis is in doubt, histopathology is similarly inconclusive (Wilhelm, Sarkamy & Calnan, 1958; Lever & Schaumburg-Lever, 1975). This is due, in part, to the conflicting data which exists on the nature of the inflammatory responses in ACD and ICD. Many studies have shown that ACD is a delayed type hypersensitivity reaction involving primarily lymphocytes and cells of the monocyte-macrophage series (Jidoi *et al.*, 1974; Silberberg, Baer & Rosenthal, 1976). However, several authors have reported also the presence of basophils as a major component of the dermal infiltrate (Medenica & Rislenberg, 1971; Dvorak & Mihm, 1972; Groth, 1978).

The polymorphonuclear leukocyte has been described as the principle infiltrating cell in ICD, particularly in animal studies (Medenica & Rislenberg, 1971; Jidoi, Kitano & Urabe, 1974). However, recent studies in humans have not confirmed this but have indicated that, as in ACD,

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the predominant cell type is the mononuclear cell, and in particular the T lymphocyte (Reimato *et al.*, 1981; Ranki *et al.*, 1983).

The role of the Langerhans cell in the development of ACD, first proposed by Silberberg (1971), is now well established. Apposition of Langerhans cells to lymphocytes, particularly in the epidermis, is believed to be evidence of antigen presentation in the early stages of ACD (Silberberg, 1973; De Panfilis *et al.*, 1983). However, apposition has been observed in a number of other conditions including mycosis fungoides (Rowden & Lewis, 1976) atopic dermatitis (Uno & Hanifin, 1980) and normal skin subjected to occlusion (Lindberg & Forslind, 1981). Langerhans cell apposition has also been described in the lesions of ICD (Kanerva, Ranki & Lauharanta, 1984).

The present study was designed to further characterize the cellular infiltrates of ACD and ICD over a prolonged time period (3 h to 7 days). Benzalkonium chloride was chosen as the irritant because of its very low allergenic potential. Observations were made at both light and electron microscopical level, with specific immunocytochemical labelling for the identification of T lymphocyte subsets and cells expressing OKT6 reactivity. By using the same patients for concurrent irritant and allergen patch testing it was hoped to eliminate patient to patient variability, enabling direct qualitative and semi-quantitative comparisons to be made.

## METHODS

Seventeen, non-atopic, healthy, adult patients took part in this study. Each had a known ACD, confirmed by previous patch testing, to one of a wide variety of allergens (Table I).

TABLE I. Allergens used in patch tests on each patient, and times of biopsy after patch test application

| Patient code no. | Allergen | Concentration (%) | Times of biopsy (h) |
|---|---|---|---|
| 1 | Phosphorus sesquisulphide | 0·5 | 3, 6 |
| 2 | Disperse yellow | 1 | 12, |
| 3 | Thiuram mix | 8 | 24, 48 |
| 4 | Fragrance mix | 16 | 96, 168 |
| 5 | Chromate | 0·5 | 3, 6 |
| 6 | Epoxy resin | 1 | 12 |
| 7 | Chromate | 0·5 | 24, 96 |
| 8 | Chromate | 0·5 | 3, 6 |
| 9 | Thiuram mix | 8 | 12, |
| 10 | Chromate | 0·5 | 12, 96 |
| 11 | Cinnamaldehyde | 2 | 24, 48 |
| 12 | Fragrance mix | 16 | 168 |
| 13 | Fragrance mix | 16 | 24, 96 |
| 14 | Ethylene diamine | 1 | 48 |
| 15 | Chromate | 0·5 | 48, 168 |
| 16 | Thiuram mix | 0·5 | 168, |
| 17 | Neomycin | 20 | 3, 6 |

A total of four ACD biopsies and four ICD biopsies were obtained at each time point.

### *Epicutaneous patch tests*

Patch tests were carried out using occlusive Finn Chambers, with one biopsy being taken from each patch test site. Standard concentrations of allergens prepared in soft paraffin (Trolab, Hermal-Chemie, Reinbek, F.R.G.) were used to reproduce ACD at one or two sites on the volar aspect of the right forearm of each patient. ICD was induced by applying 0·5% benzalkonium chloride simultaneously to the left forearm. This irritant had been chosen after a pilot study, to produce an irritant erythema without necrosis or pustulation. All patch tests were removed at the time of biopsy or after a maximum of 48 h application.

### *Biopsies*

Elliptical skin biopsies were taken from the patch test sites 3, 6, 12, 24, 48, 96 and 168 h after application of the patch test using 2% lignocaine as local anaesthetic. Irritant sites were always biopsied simultaneously with allergic sites in each patient. Times of biopsy were randomized between patients, although for convenience the 3 and 6 h samples were taken from the same four individuals. The skin samples were trisected immediately and prepared for microscopy.

### *Histology*

Biopsy samples were fixed in 10% formaldehyde, dehydrated in acetone and embedded in Transmit-LM epoxy resin (Taab Laboratories Ltd, Reading, Berkshire, U.K.). 1 μm sections were cut, etched with sodium ethoxide for 5 min, and stained with either haematoxylin and eosin or Giemsa stain prior to examination. In addition, toluidine blue stained semi-thin sections (1 μm) obtained from the EM samples, and haematoxylin and eosin stained frozen sections (5 μm) were examined.

### *Histological evaluations*

Three sections, obtained from different areas of each biopsy, were examined blind by two independent observers. In addition to a qualitative appraisal of each biopsy, a semi-quantitative method was used to compare allergic and irritant responses. Histopathological features were assessed on a numerical scale: spongiosis (0–4), intracellular oedema (0–2), exocytosis (0–2), acanthosis (0–2), hypoplasia (0–1), and dermal infiltration (0–6). A mean total pathology score was calculated for each biopsy. Irritant and allergen biopsy scores for each patient were compared using Wilcoxon's matched-pairs signed-ranks test for non-parametric data.

### *Immunocytochemistry*

*Freezing and storage of tissue.* Immediately after biopsy, skin samples were embedded in OCT (R.A. Lamb, London, U.K.) and snap frozen in liquid nitrogen. Specimens were stored under isopentane at −70°C until required.

*Monoclonal antibodies.* Monoclonal antibodies produced by Ortho Diagnostics (High Wycombe, Bucks, U.K.) were used to identify Langerhans cells (OKT6), T-helper-inducer cells (OKT4), and T-suppressor/cytotoxic cells (OKT8).

*Immunoperoxidase staining.* An indirect labelling technique was used. Cryostat sections (4 μm) were air dried for 30 min, fixed in acetone for 15 min at 4°C and treated with 10% normal rabbit serum for 30 min to reduce non-specific binding. The sections were then incubated for 30 min with the primary monoclonal antibody. OKT4 was applied undiluted whilst OKT6 and OKT8 were diluted 1:4 in phosphate buffered saline (PBS) containing 10% normal human serum and 10% bovine serum albumin. The sections were washed in PBS and incubated for 30 min with peroxidase conjugated rabbit anti-mouse immunoglobulin (Dako Ltd, High Wycombe, Bucks, U.K.) diluted 1 : 9 in the same diluent as that used for the primary antibodies. The sections were then further washed in PBS and the peroxidase reaction developed using 0·05% diaminobenzidine containing 0·003% $H_2O_2$ as substrate. Sections were counterstained with Mayer's haematoxylin and mounted in DPX.

Positive controls were performed using normal tissue from lymph nodes and tonsils, and negative controls were included at all stages.

*Estimation of T helper/T suppressor cell ratio.* Immunoperoxidase labelling with OKT4 and OKT8 was carried out on serial sections from three different areas of each biopsy. The numbers of positively stained cells per section were estimated and the mean ratios of helper to suppressor T lymphocytes calculated.

### *Electron microscopy*

Biopsy samples for electron microscopy were fixed in 3% glutaraldehyde in 0·1 M phosphate buffer, pH 7·2, for 4 h at room temperature, rinsed in phosphate buffer and post-fixed in 1% osmium tetroxide in 0·1 M phosphate buffer for 1 h at room temperature. Following several

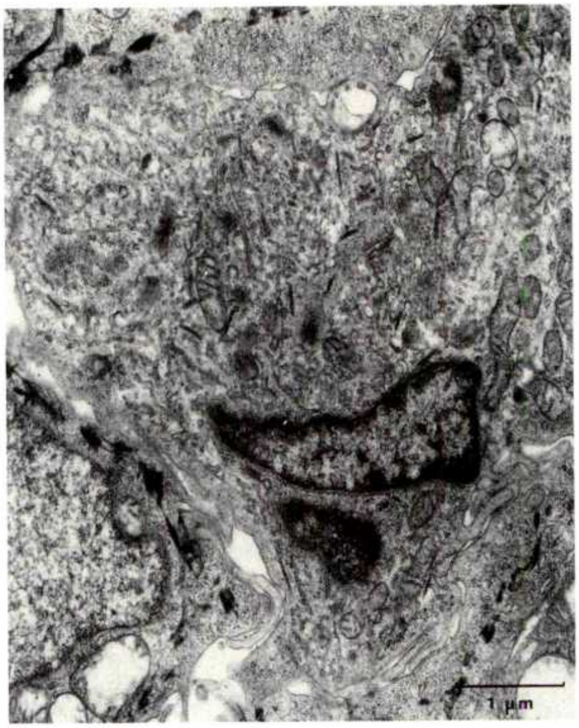

FIGURE 1. Epidermal Langerhans cell showing evidence of metabolic activity with distended endoplasmic reticulum and plentiful Birbeck granules and mitochondria (Patient 1; irritant site, 6 h). (Original × 26 000).

washes in distilled water, the samples were treated en bloc with 1% aqueous uranyl acetate at 4°C overnight, dehydrated in graded acetones and embedded in Araldite Premix resin (Taab Laboratories Ltd, Reading, Berkshire, U.K.).

Semi-thin sections (1 μm) were prepared for orientation purposes and light microscopy. Thin sections (60 nm) were cut using an Ultracut microtome (Reichert-Jung), stained with lead citrate and examined using a Philips 400T transmission electron microscope operated at 100 kV.

## RESULTS

Skin biopsies taken from both irritant and allergen sites 3 h after patch testing appeared normal. There was no evidence of oedema or cellular infiltration and the Langerhans cells were of typical appearance and distribution.

Initial cellular changes were observed after 6 h by electron microscopy. In both allergen and irritant samples there appeared to be an increase in the numbers of basal and suprabasal epidermal Langerhans cells, although this was assessed qualitatively only. There was morphological evidence of increased metabolic activity in some of these cells, including distended endoplasmic reticulum, pronounced microtubules and an increase in the numbers of Birbeck granules and mitochondria (Fig. 1). Occasionally, Langerhans cells were necrotic with few organelles, condensed chromatin and shrunken cytoplasm, particularly in the perinuclear region (Fig. 2). Small numbers of lymphocytes were present in the basal epidermis of some

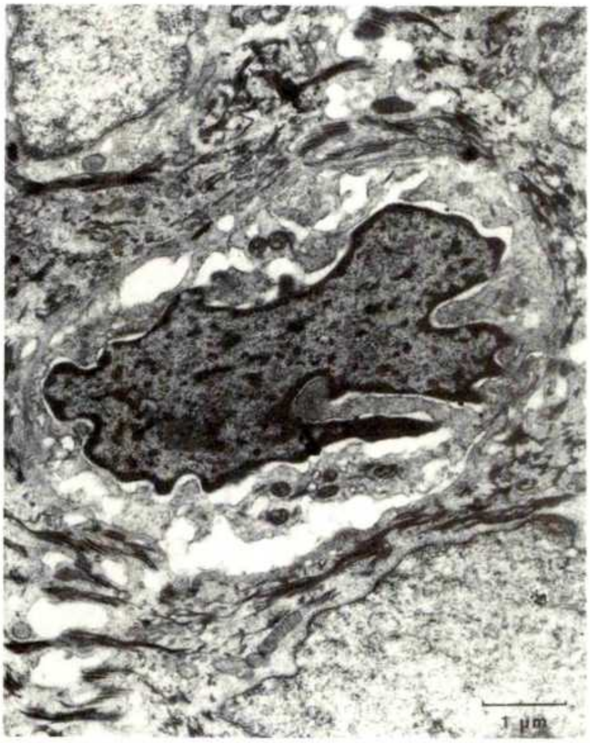

FIGURE 2. Necrotic epidermal Langerhans cell with condensed chromatin and shrunken cytoplasm (Patient 5; allergic site, 6 h). (Original × 20 000).

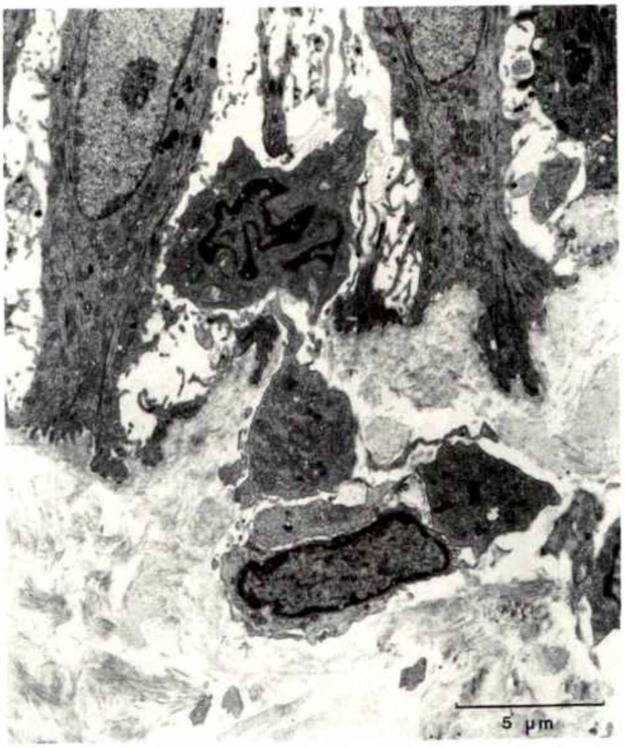

FIGURE 3. Migration of lymphocyte through the dermo-epidermal junction (Patient 8; allergic site, 6 h). (Original × 7200).

patients (Fig. 3). Both allergic and irritant biopsies showed occasional sites of Langerhans cell/lymphocyte apposition in the epidermis (Fig. 4).

In addition to the small influx of lymphocytes into the epidermis, large OKT6 positive mononuclear cells of dendritic or fibroblastic appearance were observed immediately below the dermo-epidermal junction in the majority of patients (Fig. 5).

By 12 h after patch test application, varying degrees of epidermal spongiosis and oedema had developed, with increased exocytosis of lymphocytes into the basal, suprabasal and occasionally the upper epidermis. Langerhans cell numbers were similar to those observed at 3 h and the cells were of characteristic appearance, remaining so for the rest of the test period. Perivascular accumulations of small numbers of mainly OKT6 positive cells were seen in the dermis.

The development of the ICD and ACD lesions after 12 h was extremely variable, both in the extent of epidermal damage and the degree of cellular infiltration. However, peak responses to allergen occurred generally between 48 and 96 h, whilst irritant reactions were maximal between 24 and 96 h. Considerable epidermal vesiculation developed in several allergic lesions after 96 h, with large OKT6 positive mononuclear cells present in the intercellular spaces and within small bullae (Fig. 6). By electron microscopy these cells resembled closely Langerhans cells, although no Birbeck granules were found in the cytoplasm despite the examination of serial sections. The integrity of the dermo-epidermal junction was always maintained, however, even in cases of severe epidermal oedema.

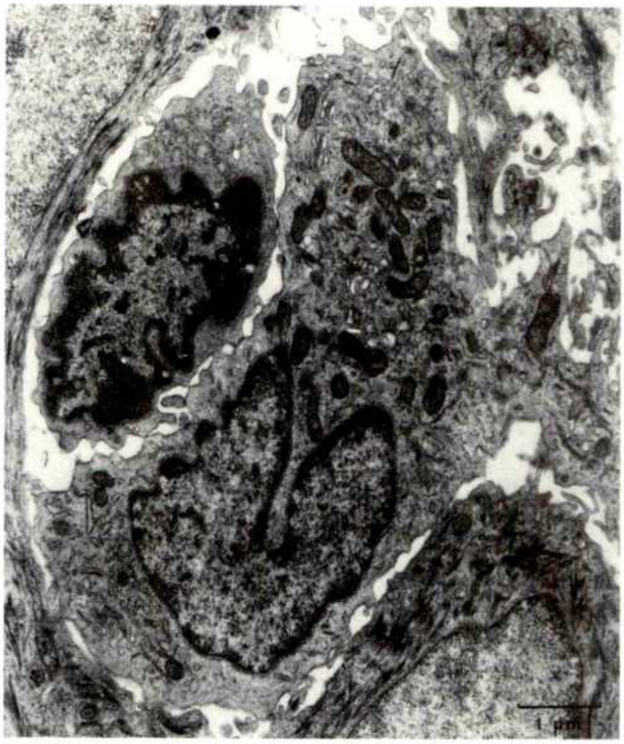

FIGURE 4. Apposition of Langerhans cell to lymphocyte in the epidermis (Patient 5; irritant site, 6 h). (Original × 20 000).

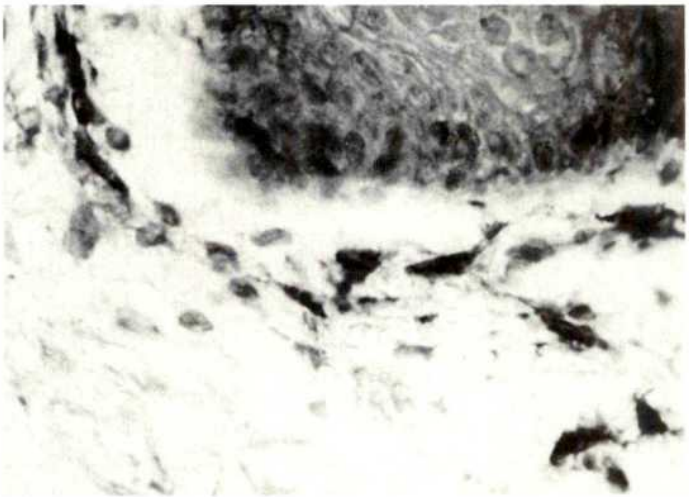

FIGURE 5. Immunoperoxidase-labelled OKT6 positive mononuclear cells immediately below dermo-epidermal junction (Patient 17; irritant site, 6 h). (Original × 800).

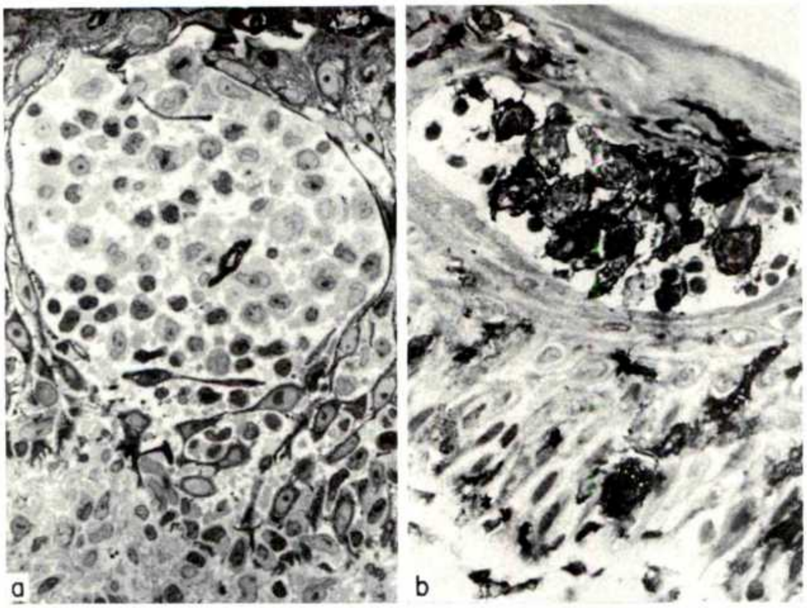

FIGURE 6. (a) Intraepidermal bulla (Patient 4; allergic site, 96 h) containing mononuclear cells closely resembling Langerhans cells. (Toluidine blue stained 1 μm resin section.) (b) The majority were OKT6 positive but were shown by electron microscopy to be devoid of Birbeck granules. (Original × 800).

The degree of dermal infiltration varied considerably between patients, but was generally greatest where extensive epidermal damage had occurred. Although there were obvious quantitative differences between these infiltrates, qualitatively they were very similar. Polymorphonuclear leukocytes, including basophils, were not present in significant numbers, except in two patients who developed *Staphylococcus aureus* infections (Patients 15 and 16). The predominant responding cell type in both ACD and ICD lesions was the T lymphocyte. Helper/suppressor ratios varied greatly between patients and within time periods, with no detectable pattern to distinguish allergic from irritant dermatitis (Fig. 8). Large mononuclear OKT6 positive cells formed a varying proportion of the dermal infiltrates (10–30%), occurring both singly and in association with perivascular collections of lymphocytes (Fig. 7). Again, despite the morphological similarities to Langerhans cells, no Birbeck granules could be detected within the cytoplasm of these cells by electron microscopy.

By 168 h after patch testing there was evidence of skin repair and recovery, particularly of the epidermis. Areas of lymphocytic infiltration in the dermis persisted in most lesions, however.

Considerable variability in the intensity of reaction to irritant and allergen occurred within individuals. These quantitative differences were compared statistically by applying Wilcoxon's test to the total pathology scores calculated for each biopsy. There was no statistically significant difference between the pathology scores for allergic and irritant sites. A graph of total pathology score per biopsy against time illustrates the large scatter and variability of responses to allergen and irritant (Fig. 9).

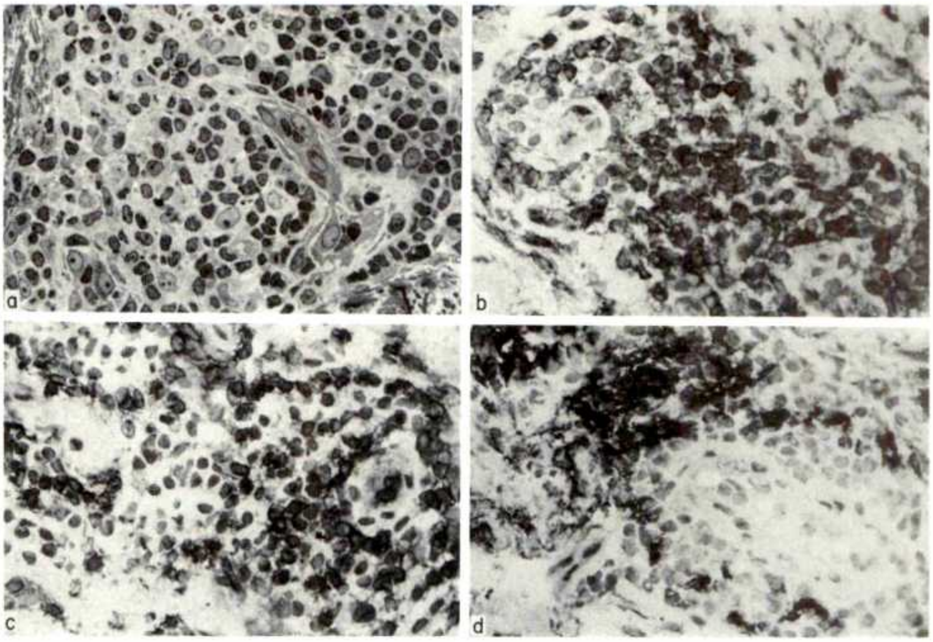

FIGURE 7. Dermal infiltrate (Patient 4; allergic site, 96 h) containing primarily lymphocytes with a small proportion of large mononuclear cells (a). (Toluidine blue stained 1 μm resin section.) Immunoperoxidase labelling of serial sections demonstrates distribution of OKT4 positive helper T lymphocytes (b); OKT8 positive suppressor T lymphocytes (c), and OKT6 positive mononuclear cells (d). (Original × 800).

## DISCUSSION

The aim of the present study was to investigate whether there are any histopathological differences between allergic contact dermatitis and irritant contact dermatitis which may be of diagnostic value. Particular emphasis was placed on the characterization of the infiltrating cells and on the examination of Langerhans cells in the early stages of inflammation. Our results showed no differences between ACD and ICD, either in the responding cell types or in the sequence of cellular changes. Quantitative differences between allergen and irritant within individuals were not statistically significant. This supports the recent findings of other workers who also failed to differentiate the two conditions using similar techniques (Reimato *et al.*, 1981; Ranki *et al.*, 1983; Kanerva *et al.*, 1984).

The standard concentrations of allergens used in the patch tests were those recommended by the I.C.D.R.G. at the time of the study. It is recognized that three of these allergens, chromate, cinnamaldehyde and fragrance mix, may also be irritant at these concentrations. However, reproducible patch tests, clinically and morphologically resembling ACD, were obtained from all the patients in whom these allergens were used.

The Langerhans cell is known now to play a key role in the afferent limb of ACD. However, there are a number of studies which suggest its involvement in the pathogenesis of other conditions such as lichen planus (Bhan *et al.*, 1981), atopic dermatitis (Uno & Hanifin, 1980) and

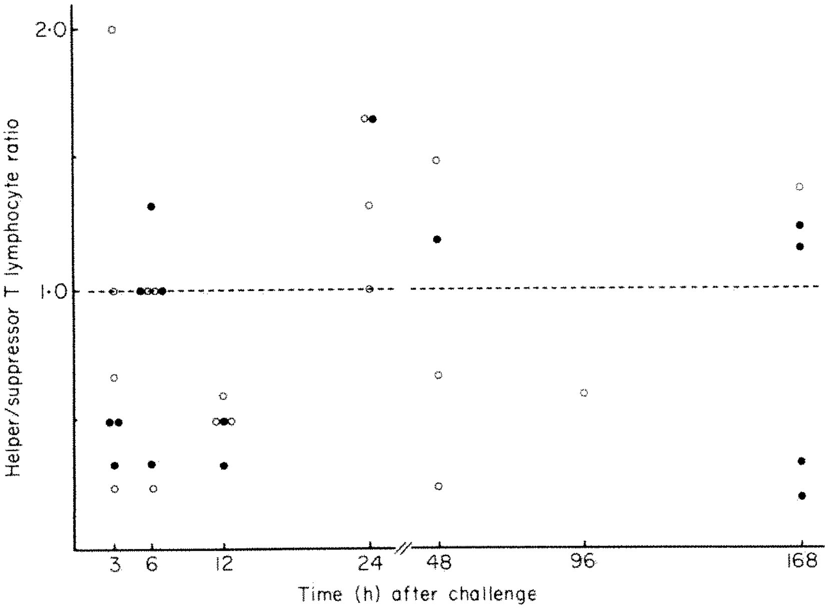

FIGURE 8. Helper (OKT4 positive) to suppressor (OKT8 positive) T lymphocyte ratios estimated in the dermal infiltrates of ACD and ICD at different time intervals after patch test application. (Several biopsies were excluded from the above due to diffuse or non-specific immunoperoxidase labelling which prevented accurate estimation of cell numbers.) ○ Irritant; ● allergic.

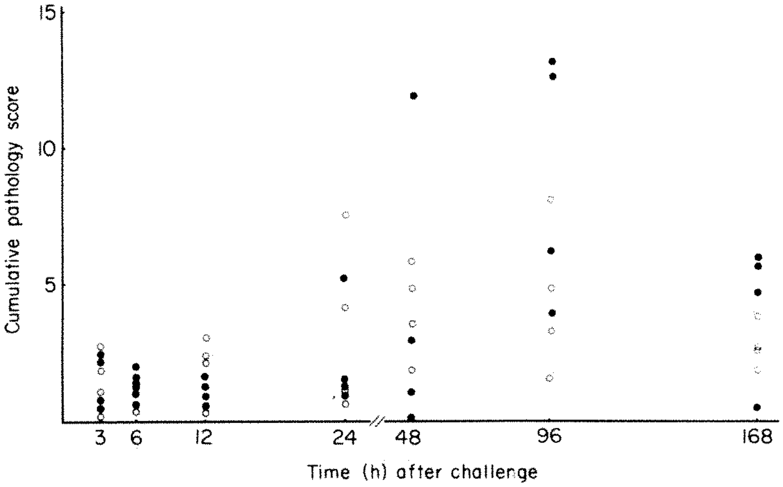

FIGURE 9. Time course of pathological changes following allergen and irritant application. ○ Irritant; ● allergic.

mycosis fungoides (MacKie, 1982). Histological evidence from our study and those of other workers (Reimato *et al.*, 1981; Kanerva *et al.*, 1984) indicates that it is also involved in ICD, undergoing morphological changes and occurring in apposition to lymphocytes in the epidermis. The functional significance of this is, as yet, unclear. It is interesting to note that in our study Langerhans cells, as identified ultrastructurally by the presence of Birbeck granules, were never seen in the dermis, except in association with hair follicles and endocrine sweat glands. Large, mononuclear, OKT6 positive cells closely resembling Langerhans cells were seen frequently, however, either in isolation or associated with lymphocytic accumulations. It is uncertain whether these cells represent migrating or recruited Langerhans cells which have lost their Birbeck granules, or whether they are macrophages/indeterminate dendritic cells. Further study, possibly using ultrastructural immunocytochemistry, is clearly required to characterize these cells fully.

The major responding cell type in both ACD and ICD lesions of our patients was the T lymphocyte. Basophils and other polymorphonuclear leukocytes were not found in significant numbers except where infection was present. Several of the studies suggesting the importance of basophils in the early stages of inflammation have been performed in animals, and it is possible that there is considerable species variation in the trigger mechanism(s) of ACD. There may also be species-related differences in cellular reactions to irritants. Polymorphonuclear leukocytes are a common feature of ICD in experimental animals, particularly the guinea-pig, but have rarely been described in human ICD. The chemical nature of the irritant, and the concentration at which it is applied, may also be important factors in determining the appearance of polymorphonuclear leucocytes.

Considerable significance is attached to the relationship between helper and suppressor T lymphocytes in cellular infiltrates. In our study there was no detectable pattern of lymphocytic infiltration which could be used to distinguish ACD from ICD. The ratio of helper to suppressor T lymphocytes varied enormously between patients, between different ACD and ICD sites, and within time periods. However, the small sample sizes in some groups made firm conclusions difficult to draw.

There are still many aspects of the initiation and development of ACD and ICD which are poorly understood. In particular, the Langerhans cell appears to be involved not only in ACD but also in ICD and a wide range of other skin reactions. Further work is required, directed towards a fuller understanding of the responding cell types in ACD and ICD, using ultrastructural immunocytochemistry in conjunction with a broader spectrum of monoclonal antibodies.

## ACKNOWLEDGMENTS

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# Fine needle aspiration cytology in dermatology: a clinicopathological appraisal

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## SUMMARY

The possible clinical value of fine needle aspiration cytology (FNAC) has been assessed prospectively in 60 patients presenting with cutaneous lesions. The cytological diagnosis in each case has been compared with the clinical diagnosis and biopsy result. The principal potential use appears to be the rapid confirmation of the clinical diagnosis of basal cell carcinoma to allow immediate referral for radiotherapy or plastic surgery. FNAC could also prove useful when the clinical diagnosis of malignant melanoma is in doubt and primary diagnostic excision is difficult or disfiguring. Accurate distinction could usually be made between benign and malignant lymphoproliferative conditions, but further classification was difficult. Metastatic malignancy could be diagnosed with ease and other characteristic cytological appearances were seen with naevocellular naevi, pyogenic granuloma and pilar cysts. However, there were limitations in achieving an accurate diagnosis in approximately half the cases, and consequently, FNAC cannot be regarded in general as a substitute for histological diagnosis.

The value of cytodiagnosis in dermatology has been the subject of several previous studies. Dudgeon & Patrick (1927) found that basal cell carcinoma could be diagnosed accurately using cytology, and this has been confirmed by other observers (Urbach, Burke & Traenkle, 1957; Brown, Klaber & Robertson, 1979). In addition, cytological examination of blisters, as introduced by Tzanck, is well recognized to be useful in the rapid demonstration of acantholytic epidermal cells in pemphigus and the viral inclusions of herpes (Graham, Bingul & Burgoon, 1963).

However, these studies have been based on cytological preparations obtained from tissue imprints or scrapings. An alternative technique, fine needle aspiration cytology (FNAC), was introduced in the 1930s by Stewart (1933) and Martin and Ellis (1934) in the U.S.A. Since the 1950s, it has been used extensively in Scandinavia, and in recent years its practice has increased rapidly in the U.K. (Griubb, 1981; Melcher, Linehan & Smith, 1984; Lever, Trott & Webb, 1985). The principal application of the technique has been by general surgeons and radiologists

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in the rapid diagnosis of abdominal, thoracic, breast, cervical and nodal malignancies. The value of FNAC in clinical dermatology has either received little emphasis (Lever, Trott & Webb, 1985) or is boldly stated to have limited use (Melcher, Linehan & Smith, 1984).

A prospective study was undertaken to assess the value of FNAC in patients presenting with cutaneous swellings in a dermatology department.

## METHODS

Sixty consecutive patients presenting with a cutaneous swelling to a single dermatologist (GR) were entered into the study. The research nature of the aspiration procedure was explained and verbal consent obtained in each case.

After aspiration, each lesion was biopsied or curetted, as clinically appropriate, and submitted for routine histopathological examination. Each cytological preparation was read 'blind' by a single histopathologist (DNS) with no knowledge of the clinical or final histopathological diagnosis. The dermatologist (GR) had no practical experience of fine needle aspiration prior to the commencement of the study.

All aspirations were performed with a 20 ml plastic disposable syringe coupled with a 21 gauge needle using a braced thumb technique (Webb, 1982). After the skin had been cleaned with ethyl alcohol, the lesion was held firmly between the thumb and forefinger of one hand. The needle was inserted into the lesion and the plunger of the syringe pulled back to exert suction. This was maintained with the thumb, and the needle moved through the lesion several times. Still with the needle in the skin, suction was released slowly. The needle was then removed from the skin and the syringe from the needle. The syringe was filled with a little air, reconnected to the needle and the contents of the needle blown gently onto one or more 76 × 26 mm glass microscope slides. The specimen was then spread with another slide held at an oblique angle, using the same technique as making a blood film. The slides were waved vigorously in the air to dry them as quickly as possible.

The aspirate was fixed in methyl alcohol for 5 min, handstained using May-Grunwald-Giemsa and mounted (Melcher, Linehan & Smith, 1984).

## RESULTS

Table 1 compares the results of fine needle aspiration cytology, histological and clinical diagnosis.

The detailed cytological findings in the different disorders were as follows:

*Naevocellular naevus.* Aspiration produced numerous grape-like clusters of cells displaying regular round or oval nuclei and possessing minimal cytoplasm. One or two small nucleoli were usually present (Fig. 1).

*Squamous cell papilloma.* These were difficult to aspirate and showed scattered, mature keratinocytes with small, regular, round nuclei and a low nuclear-cytoplasmic ratio. Occasional cells showed perinuclear vacuolation (koilocytosis) suggestive of a viral aetiology (human papilloma virus).

*Hypertrophic solar keratosis.* These were difficult to aspirate and showed scattered mature keratinocytes with small regular round nuclei. With the exception of the absence of vacuolation, the appearance was indistinguishable from a squamous cell papilloma.

TABLE 1. Results of needle aspiration cytology related to histological and clinical diagnosis

| | Needle aspiration cytology | | | | |
|---|---|---|---|---|---|
| Final histological diagnosis | *Successful | *Poor | *Failed | Diagnosis | Clinical diagnosis |
| Squamous cell papilloma (viral) | | 1 | 3 | Squamous cell papilloma (viral) | Squamous cell papilloma (viral) (4)* |
| Seborrhoeic wart | | | 4 | — | Seborrhoeic wart (4) |
| Naevocellular naevus | 2 | | | Naevocellular naevus | Naevocellular naevus |
| Hypertrophic solar keratosis | 2 | 1 | 1 | Squamous cell papilloma (3) | Solar keratosis (2)<br>Squamous cell carcinoma (1)<br>Basal cell carcinoma (1) |
| Follicular poroma | | 1 | | Squamous cell papilloma | Squamous cell papilloma (viral) |
| Pilar cyst | 2 | | | Pilar cyst | Pilar cyst |
| Epidermoid cyst | | 1 | | Epidermoid cyst | Epidermoid cyst |
| Pyogenic granuloma | 3 | | | Angioma (3) | Pyogenic granuloma (3) |
| Dermatofibroma | | | 2 | — | Dermatofibroma |
| Granuloma annulare | | | 1 | — | Granuloma annulare |
| Nonspecific folliculitis | 1 | | | Inflamed epidermoid cyst | Angiosarcoma |
| Basal cell carcinoma | 15 | 2 | 1 | Basal cell carcinoma (17) | Basal cell carcinoma (17)<br>Squamous cell carcinoma (1) |
| Squamous cell carcinoma (invasive) | 2 | | | Squamous cell carcinoma | Squamous cell carcinoma<br>Keratoacanthoma |
| Malignant melanoma | 2 | | | Malignant melanoma (2) | ? Seborrhoeic wart<br>? Pyogenic granuloma |
| Paget's disease | | 1 | | ? Adenocarcinoma | ? Paget's disease |
| Leiomyosarcoma | | | 1 | — | Soft tissue tumour |
| Malignant fibrous histiocytoma | 1 | | | Pleomorphic sarcoma | Soft tissue tumour |
| Angiosarcoma (poorly differentiated) | 1 | | | Malignant | ? Angiosarcoma |
| Malignant lymphoma (non-Hodgkin's) | 1 | | | Malignant lymphoma | Malignant lymphoma |
| Lymphocytoma cutis | 1 | | | Benign lymphoid proliferation | ? Lymphocytoma cutis<br>? Lymphoma |
| Myeloma (poorly differentiated) | 1 | | | Myeloma | ? Myeloma |
| Lymphomatoid papulosis | 1 | | | Lymphoid proliferation | ? Lymphomatoid papulosis |
| Mycosis fungoides | 1 | | | Malignant lymphoma | ? Mycosis fungoides ? Leprosy |
| Oat cell carcinoma | 1 | | | Oat cell carcinoma | ? Oat cell carcinoma |
| Hidradenoma | | | 2 | — | ? BCC (1)<br>? BCC ? sweat gland tumour |
| Spiradenoma | | | 1 | — | ? Sweat gland tumour |

* Numbers of cases.

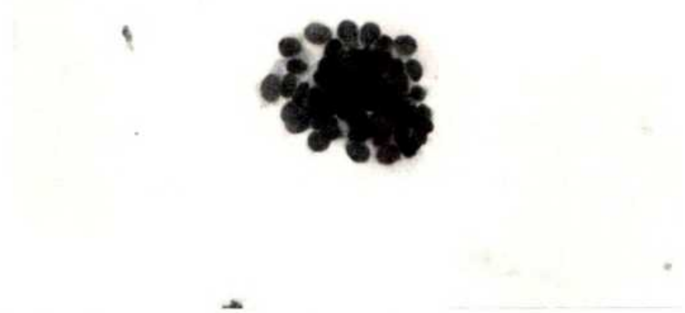

FIGURE 1. Naevocellular naevus showing grape-like clusters. Fine needle aspirate, May-Grunwald/Giemsa. (Original × 80).

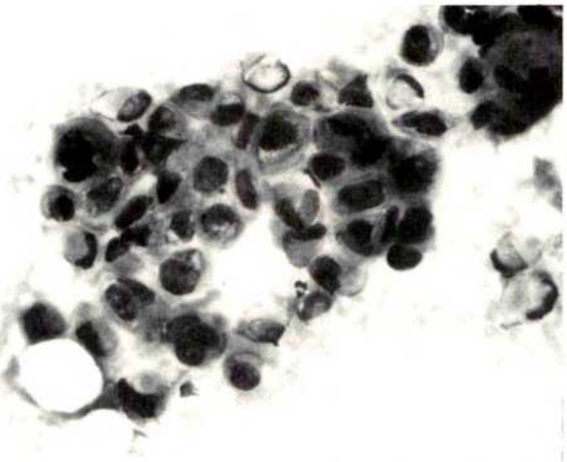

FIGURE 2. Squamous cell carcinoma showing marked nuclear pleomorphism, perinuclear vacuoles and cytoplasmic rims. (Original × 300).

*Follicular poroma*. This was difficult to aspirate and showed scattered mature keratinocytes with small regular round nuclei. With the exception of the absence of vacuolation, the appearance was indistinguishable from a squamous cell papilloma.

*Squamous cell carcinoma*. These aspirated easily and showed large clumps of cells displaying marked nuclear pleomorphism. The perinuclear area of cytoplasm often appeared pale or vacuolated in contrast to the denser peripheral rim. Occasional keratin whorls were present (Fig. 2).

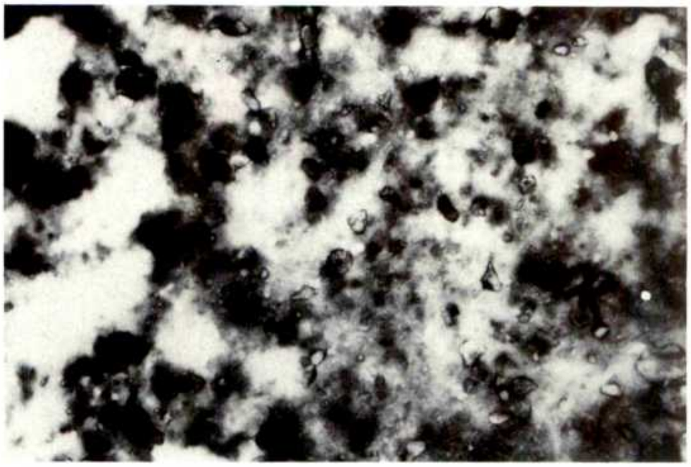

FIGURE 3. Pilar cyst showing crystalline material. (Original × 300).

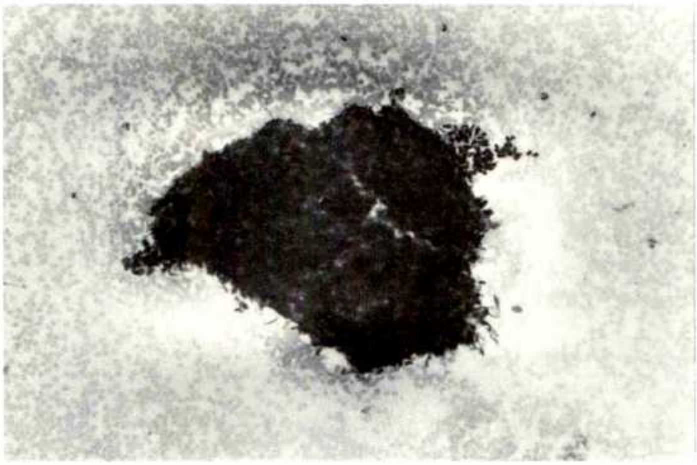

FIGURE 4. Basal cell carcinoma showing solid clump of cohesive cells. (Original × 20).

*Pilar cyst*. This aspirated easily and showed amorphous debris with birefringent crystalline material (Fig. 3).

*Epidermoid cyst*. This aspirated with difficulty and showed keratinaceous debris.

*Pyogenic granuloma*. These aspirated easily and were always severely blood-stained. Elongated vascular channels were always discernible with variable numbers of inflammatory cells.

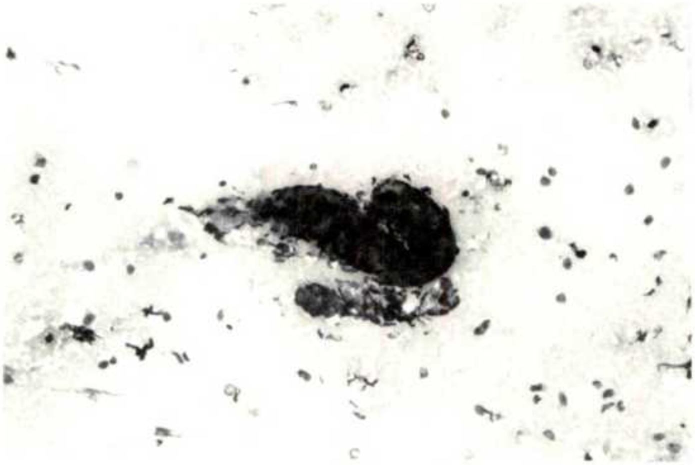

FIGURE 5. Basal cell carcinoma showing an 'elephant-trunk' clump of cells. (Original × 20).

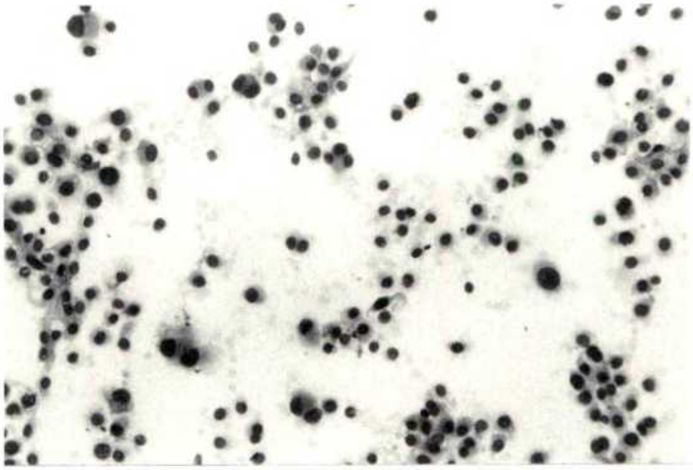

FIGURE 6. Malignant melanoma showing widely dispersed cells with variation in nuclear size. (Original × 60).

*Basal cell carcinoma.* These aspirated relatively easily and always produced extremely solid clumps of strongly cohesive cells. Only small numbers of peripheral cells appeared in monolayer form and these displayed slightly irregular and hyperchromatic nuclei with a high nuclear-cytoplasmic ratio. In many instances 'elephant-trunk' clumps of cells were present. Two biopsies showed moderate squamous keratinization, but this appeared proportionally less than the basaloid component (Figs 4 and 5).

*Malignant melanoma.* These aspirated easily and produced large numbers of widely dispersed cells. The cells were predominantly solitary but small clusters were also present. Most nuclei showed a regular round contour but with marked variation in size and nuclear chromatin

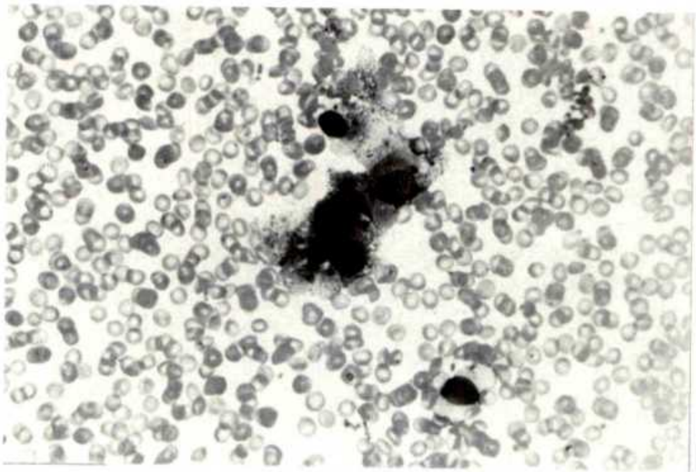

FIGURE 7. Malginant melanoma showing cytoplasmic melanin granules. (Original × 300).

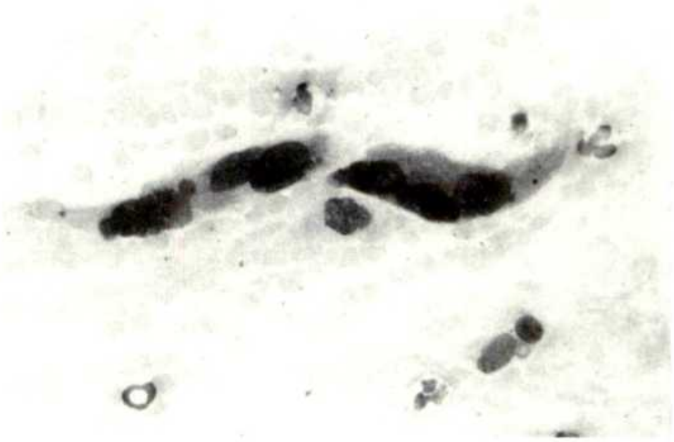

FIGURE 8. Malignant fibrous histiocytoma showing spindle multinucleate cells. (Original × 300).

distribution. Nucleoli were usually large and prominent. The nuclear-cytoplasmic ratio was low, and occasional cells displayed melanin granules (Figs 6 and 7).

*Paget's disease*. This aspirated with difficulty and showed clusters of cells displaying pleomorphic nuclei with one to three small nucleoli. Cytoplasm was present in variable amounts and occassionally appeared vacuolated.

*Malignant fibrous histiocytoma*. This aspirated easily and showed numerous elongated mesenchymal type cells. These were often multinucleate with hyperchromatic nuclear chromatin (Fig. 8).

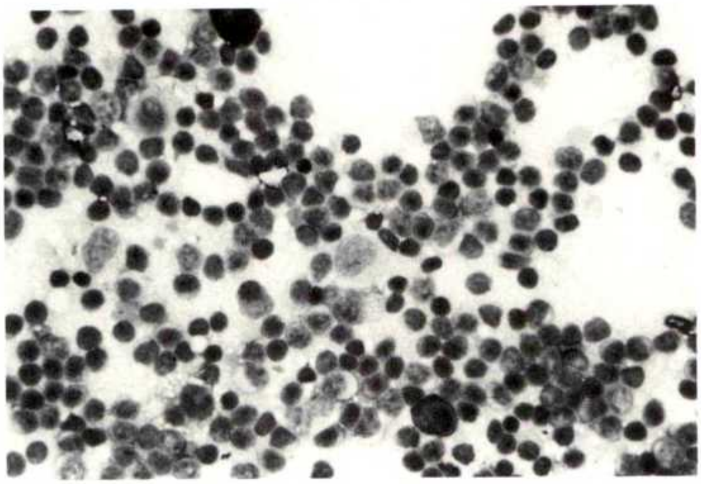

FIGURE 9. Lymphocytoma cutis showing a diverse population of lymphocytes, histiocytes and plasma cells. (Original × 90).

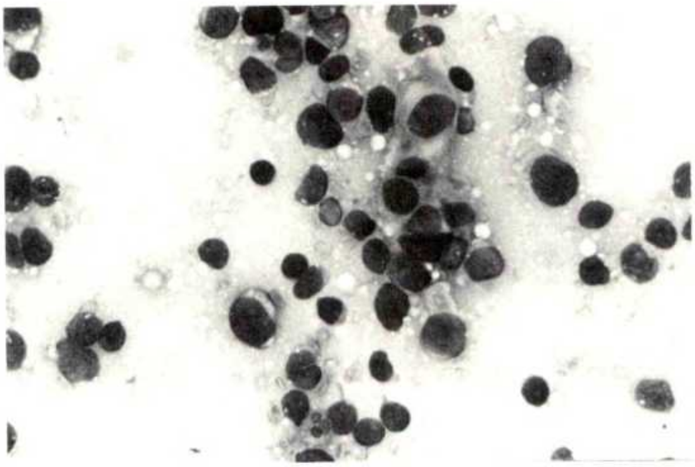

FIGURE 10. Mycosis fungoides showing non-cohesive pleomorphic haemopoietic cells. (Original × 400).

*Angiosarcoma*. This aspirated easily and showed undifferentiated malignant cells similar to the poorly differentiated nature of the tumour seen histologically.

*Malignant lymphoma (non-Hodgkin's)*. This aspirated easily and displayed a pure dual population of small and large lymphocytes. The cleaved nature of the small follicular centre cells seen histologically was not apparent cytologically.

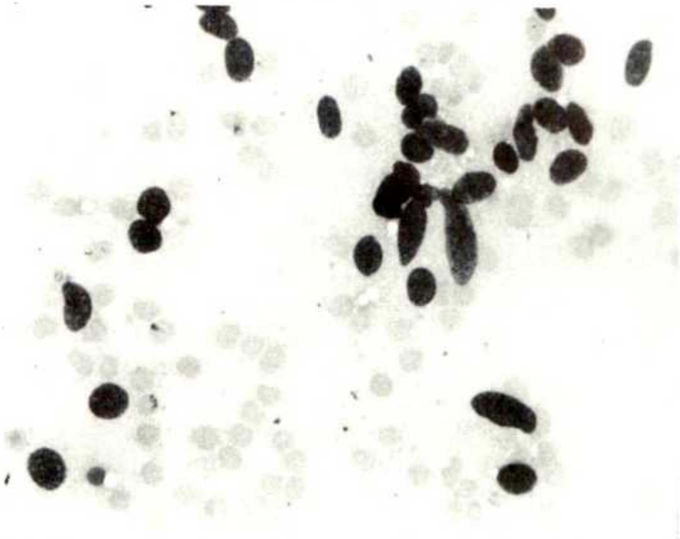

FIGURE 11. Metastatic oat cell carcinoma showing small cells with round to oblong nuclei. (Original × 300).

*Lymphocytoma cutis*. This aspirated easily and showed a widely diverse population of cells, including variable sized lymphocytes, histiocytes and plasma cells (Fig. 9).

*Multiple myeloma*. This aspirated easily and displayed a non-cohesive population of haemopoietic cells. The cytoplasm appeared basophilic and finely vacuolated, but the plasmacytoid differentiation apparent histologically was difficult to identify.

*Mycosis fungoides*. This aspirated easily and displayed a non-cohesive population of haemopoietic cells (Fig. 10). The hyperconvoluted nature of the nuclei seen histologically was not easily discernible.

*Oat cell carcinoma*. This aspirated easily and displayed numerous isolated small cells with round to oblong nuclei and a very high nuclear-cytoplasmic ratio (Fig. 11).

*Seborrhoeic wart, dermatofibroma, granuloma annulare, leiomyosarcoma, hidradenoma and spiradenoma*. These lesions failed to aspirate.

## DISCUSSION

As a clinical procedure, fine needle aspiration cytology is inexpensive, requires simple equipment and is not time-consuming. Although the patient sustains a needle prick, local anaesthesia is unnecessary and a biopsy is potentially prevented.

This study demonstrated that no previous specific technical expertise is required by the clinician and that successful aspirates can be obtained easily after only a brief period of instruction. It is evident that in some cases an accurate pathological diagnosis could be obtained within minutes whilst the patient is still in the out-patient clinic. Thus, treatment could be planned and explained to the patient before leaving the clinic. The number of repeat out-patient clinic attendances could thus be reduced and valuable time saved on biopsy lists.

The principal use of needle aspiration cytology would appear to be in the rapid diagnosis of basal cell carcinoma. No false-positives were observed in this group and the false-negative incidence was only approximately 5%, a figure broadly comparable to routine biopsy but not sufficiently low to obviate the need for routine biopsy. Twelve of the 18 cases were considered unsuitable for complete excision and were referred subsequently for either radiotherapy ($n = 11$) or plastic surgery ($n = 1$). Basal cell carcinoma is the commonest malignant skin tumour and accounts for up to 3% of patients seen by dermatologists in the U.K. (Rook, Wilkinson & Ebling, 1972). As most radiotherapists insist on a definitive pathological diagnosis prior to treatment, a large number of biopsies could potentially be obviated by aspiration cytology and thereby make a considerable saving in time and money.

These aspiration findings are in broad agreement with previous studies investigating scrape cytology and the rapid diagnosis of basal cell carcinoma (Brown, Klaber & Robertson, 1979). Although the false-negative rate was broadly similar, a false-positive rate of approximately 2% has been observed with scrape cytology; the major area of misinterpretation being the diagnosis of extensive keratinization as squamous cell carcinoma. Two aspirates in our series showed areas of squamous differentiation, but the underlying basal cell nature of the neoplasm was evident.

Both cases of squamous cell carcinoma were diagnosed accurately by aspiration cytology but, as mentioned by Melcher, Linehan and Smith (1984), possible diagnostic difficulties can be envisaged between squamous carcinoma and keratoacanthoma.

The only absolute contra-indication to needle aspiration in this study was relative clinical certainty about the diagnosis of malignant melanoma. Patients with malignant melanoma who have undergone diagnostic biopsy prior to excision are known to have a poorer prognosis and this was thought to be also probably applicable to aspiration cytology. This is unfortunate as aspirated malignant melanoma cells display a highly characteristic cytological appearance. In two cases where there was clinical uncertainty, a definitive cytological diagnosis was achieved easily, and possible differential diagnoses such as pigmented basal cell carcinoma, seborrhoeic wart and pyogenic granuloma were completely excluded. Consequently, aspiration cytology may prove useful in situations where the clinical diagnosis of malignant melanoma is in doubt and a primary excision is difficult or disfiguring.

Other lesions giving rise to characteristic cytological appearances included naevocellular naevi, pilar cysts, pyogenic granuloma and metastatic carcinoma.

Dermal lesions such as dermatofibroma and granuloma annulare could not be aspirated and the technique appeared poor in the accurate diagnosis of soft tissue sarcomas.

Cutaneous lymphoproliferative disorders aspirated with ease and, with the exception of lymphomatoid papulosis, accurate distinction was made between benign and malignant conditions. However, further classification then proved difficult, although this could probably be improved by the application of modern immunohistological techniques to the aspirate.

Despite these successes, there were limitations in the achievement of accurate diagnosis in over half of the 60 cases studied. This included a failure to aspirate ($n = 10$), wrong diagnosis ($n = 4$), and limited accuracy ($n = 10$). Consequently, FNAC of many skin lesions cannot be regarded at present as a substitute for histological diagnosis; with the possible exception of basal cell carcinoma.

## ACKNOWLEDGMENTS

We are grateful to Dr M.Kesseler and Dr A.Messenger for helpful advice.

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# A study of HLA antigen association in localized and generalized granuloma annulare

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## SUMMARY

HLA-A and -B antigen frequencies were studied in 78 patients, 35 with localized granuloma annulare and 43 with generalized granuloma annulare (GA). Twenty-eight patients in each group were also typed for HLA-DR antigens. A group of 200 healthy age-matched subjects served as controls.

HLA-A31 and B35 were increased significantly in patients with generalized GA, but not in the localized form. HLA-DR antigen distribution showed no significant variation.

There are conflicting reports on the possible association between granuloma annulare (GA) and antigens of the HLA system (Mobacken, Lindholm & Sandberg, 1979; Andersen & Verdich, 1979; Middleton & Allen, 1984). In a previous study, we have demonstrated a significant increase in the frequency of HLA-B35 in patients with generalized granuloma annulare (GGA). However, in patients with localized granuloma annulare (LGA) we did not find a significant association with any of the 26 HLA-A and HLA-B antigens tested (Friedman-Birnbaum *et al.*, 1978).

We have now tested a larger group of patients with both LGA and GGA. In addition, we have examined the possibility of an association between HLA-DR antigens and both types of GA.

## METHODS

Thirty-five patients with LGA (25 female, 10 male) and 43 with GGA (38 female, 5 male) were tissue typed for 34 HLA-A and HLA-B antigens. Twenty-eight patients in each group were also typed for HLA-DR antigens.

Most patients (30 of 35 with LGA, 38 of 43 with GGA) were Ashkenazi Jews of Eastern European origin. The majority of these patients have been described previously (Friedman-Birnbaum, Bergman & Abraham, 1984). The diagnosis of GA was confirmed in all cases by

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histological examination. In cases with GGA, at least two biopsies from two different sites were performed. Cases in which the number and extent of the lesions were borderline and did not permit a definite classification in one of the two clinical types of the disease were excluded from the study. The control group consisted of 200 apparently healthy subjects, who were matched for age and ethnic origin and were not related to the patients. Tissue typing for HLA-A and -B antigens was performed using the microlymphocytotoxicity test, National Institute of Health (NIH) standard technique (Ray *et al.*, 1974). Typing sera were obtained from the NIH Serum Bank.

HLA-DR typing was performed on nylon wool-enriched B lymphocytes using the extended incubation microlymphocytotoxicity testing procedure (Terasaki *et al.*, 1978).

Statistical analysis was carried out using a chi square test for two independent samples, incorporating Yates correction (Svejgaard & Ryder, 1979; Friedman-Birnbaum *et al.*, 1978).

## RESULTS

The frequency of HLA-A, HLA-B and HLA-DR antigens in the two groups of patients and in the control group is shown in Tables 1 and 2.

The frequency of HLA-A31 was increased in patients with GGA as compared with its frequency in both controls and patients with LGA. The difference in frequency between patients with GGA and controls remained significant even after correction for the number of antigens tested ($P < 0{\cdot}01$).

HLA-B35 frequency was increased in patients with GGA as compared with both patients with LGA and controls ($P < 0{\cdot}01$ and $P < 0{\cdot}0002$ respectively). The frequency of HLA-B35 was also increased in patients with LGA; however, this difference was not statistically significant when corrected for the number of antigens tested. The frequency of HLA-DR antigens did not differ in the various groups tested except for a decreased incidence of HLA-DR4 in patients with LGA as compared with its frequency in patients with GGA and with that in controls. However, this difference was not statistically significant when corrected for the number of antigens.

## DISCUSSION

This study confirms our earlier findings on a small group of patients of an increased frequency of HLA-B35 in GGA patients compared to controls and LGA patients (Friedman-Birnbaum *et al.*, 1978). In addition, the present study reveals an increased frequency of HLA-A31 in patients with GGA as compared with its frequency in the controls and LGA patients. This difference remained significant after correction for the number of antigens tested.

It should be noted that in our earlier study we also found an increased frequency of HLA-Aw31 (now A31) in patients with GGA. However, the difference in its frequency as compared to that of LGA patients and controls was not then statistically significant, probably because of the relatively small group tested.

Our previous work on the HLA antigen frequencies of locus A and B in patients with LGA showed no significant difference when compared to their frequencies in controls. This is in accordance with the findings of Mobacken, Lindholm and Sandberg (1979) in a group of Swedish LGA patients. In the study of Middleton and Allen (1984), HLA-A29 was increased significantly in a group of patients with LGA from the Belfast area. This antigen was also increased in the Swedish group but the increase was not significant when correction was made

TABLE 1. Frequencies of HLA-A and HLA-B antigens in patients with localized granuloma annulare (LGA), generalized granuloma annulare (GGA) and normal controls

| Antigen | % of subjects<br>Controls ($n=200$) | <br>LGA ($n=35$) | <br>GGA ($n=43$) |
|---|---|---|---|
| HLA-A1 | 28·5 | 22·8 | 27·9 |
| HLA-A2 | 32·5 | 28·6 | 32·6 |
| HLA-A3 | 15·5 | 25·7 | 18·6 |
| HLA-A9 (A23) | 2 | 2·9 | 2·3 |
| HLA-A9 (A24) | 28 | 37·1 | 32·6 |
| HLA-A10 (A25) | 0 | 0 | 0 |
| HLA-A10 (A26) | 26 | 20 | 23·3 |
| HLA-A11 | 13·5 | 14·3 | 4·65 |
| HLA-A28 | 11 | 5·7 | 6·98 |
| HLA-A29 | 6·5 | 2·9 | 4·65 |
| HLA-A30 | 6 | 8·6 | 6·98 |
| HLA-A31 | 2·5 | 0 | 16·3$^{a}$ |
| HLA-A32 | 3·5 | 2·9 | 0 |
| HLA-Aw33 | 2 | 0 | 4·65 |
| HLA-B5 | 12 | 2·9 | 4·65 |
| HLA-B7 | 10·5 | 8·6 | 4·65 |
| HLA-B8 | 11·5 | 17·1 | 4·65 |
| HLA-B12 (B44) | 11 | 11·4 | 4·65 |
| HLA-B12 (B45) | 0 | 0 | 2·5 |
| HLA-B13 | 7·5 | 2·9 | 4·65 |
| HLA-B14 | 18·5 | 17·1 | 9·3 |
| HLA-B15 | 3 | 5·7 | 4·65 |
| HLA-B16 (B38) | 2·4 | 20 | 23·3 |
| HLA-B16 (B39) | 1 | 0 | 2·3 |
| HLA-B17 | 13 | 8·6 | 9·3 |
| HLA-B18 | 11·5 | 8·6 | 4·65 |
| HLA-B21 (B49) | 4 | 2·9 | 2·3 |
| HLA-B21 (Bw50) | 3·5 | 2·9 | 0 |
| HLA-Bw22 | 4·5 | 2·9 | 2·3 |
| HLA-B27 | 3·5 | 2·9 | 0 |
| HLA-B35 | 35·5 | 60 | 95·4$^{b,c}$ |
| HLA-B37 | 0·5 | 0 | 0 |
| HLA-B40 | 4·5 | 0 | 0 |
| HLA-Bw41 | 8 | 2·9 | 4·65 |

Statistical analysis, after correction for number of antigens compared. $^{a}$ Comparison with control $P<0·01$; relative risk 7·6. Comparison with LGA NS. $^{b}$ Comparison with control $P<0·0002$; relative risk 37·25. $^{c}$ Comparison with LGA $P<0·01$; relative risk 13·7.

TABLE 2. Frequences of HLA-DR antigens in patients with localized granuloma annulare (LGA) generalized granuloma annulare (GGA) and normal controls

| | % of subjects | | |
|---|---|---|---|
| Antigen | Control ($n=70$) | LGA ($n=28$) | GGA ($n=28$) |
| HLA-DR1 | 20 | 10·7 | 0 |
| HLA-DR2 | 20 | 17·9 | 14·3 |
| HLA-DR3 | 4·3 | 0 | 10·7 |
| HLA-DR4 | 60 | 28·6 | 60·7 |
| HLA-DR5 | 41·4 | 57·1 | 53·4 |
| HLA-DRw6 | 4·3 | 3·6 | 0 |
| HLA-DR7 | 32·9 | 46·4 | 39·3 |

for the number of antigens tested. The findings of Andersen and Verdich (1979) of an increased frequency of HLA-B8 in Danish patients with LGA, and in another group, of HLA-B15 in patients with GGA, could not be confirmed in the present study nor in that of Mobacken, Lindholm and Sandberg (1979). HLA-B14 and -B15 were not significantly increased in patients with LGA reported by Middleton and Allen (1984).

The conflicting results of HLA antigen frequencies in patients with GA in reports from different areas of the world may be explained by previous observations that the association between diseases and the various HLA genes is population specific and not universal (Brautbar *et al.*, 1982).

No strong association between DR antigens and GA could be demonstrated in the present study. The finding of a decreased incidence of HLA-DR4 in patients with LGA, that was observed before correction for the number of antigens tested, warrants further study in a larger group of patients.

The findings of Andersen and Verdich (1979) of an increased frequency of HLA-B8 in their patients with LGA and -B15 in another group with GGA is, as far as we know, the only report in support of a linkage between antigens of HLA-A and -B loci and a possible association of either form of GA with diabetes. The results reported here, like those of our earlier study, are not in favour of such an association. We have not found a higher frequency of either DR3 or DR4, alleles which confer an enhanced risk for insulin-dependent diabetes mellitus (IDDM) (Foster, 1983). Similarly, the frequency of antigen DR2, which has been called a 'protective' antigen for diabetes (Foster, 1983), was not decreased in either group of patients tested. This is of interest because more than 60% of our patients with GGA have insulin-dependent diabetes, non-insulin dependent diabetes, or an impaired glucose tolerance test.

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# Animal models for percutaneous penetration: assessing contamination of the experimental system

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## SUMMARY

We have used both quantitative and qualitative procedures to assess the validity of percutaneous penetration data obtained from the guinea-pig *in vivo*. Our results indicate that, under certain, not unusual, experimental conditions the results may be misleading and should be viewed with caution. Contamination of the experimental system, either by simple desquamation from the site of application, or trauma to the site such as rubbing or scratching, may greatly influence the results. We conclude that simple control experiments similar to those described should be conducted when using *in vivo* animal models for studies on percutaneous penetration, and particularly where the subject is not totally immobilized.

Numerous attempts have been made by workers in the field of percutaneous penetration to find a direct, predictive correlation between percutaneous penetration in man and animal models (Wester & Maibach, 1975). To this end, a variety of animals have been tested, including monkeys (Wester & Maibach, 1976), dogs, rabbits and guinea-pigs (Coutheoux, Wepierre & Marty, 1985), rats (Bartek, Budde & Maibach, 1972), and mice. In this communication we report a probable source of error of methodology when using the guinea-pig as an experimental model, although it should be noted that the experiments described are pertinent to other small animal species.

The guinea-pig typically exhibits a small post auricular bald area extending approximately one centimetre distally from the base of the ear (Zacheim & Langs, 1962). The apparent lack of fur, the covenient size, and the ease of location has made this a prime site for topical applications with a view to studying percutaneous absorption/penetration.

In a number of studies, conducted by the authors, and designed to test the effect of vehicle formulations on drug penetration where, due to the enhancing properties of occlusive materials, the site could not be covered, the post auricular bald spot was chosen as the site of application.

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Because the site was not covered, the results were subject to two possible sources of error: (a) scratching and rubbing of the site by the animal such that the dose was either ingested or knocked to the bottom of the cage; (b) desquamation—natural loss of corneocytes to the bottom of the cage, which either contained or were coated in the drug to be studied.

In an effort to elucidate further the problems of contamination in this *in vivo* system, two large molecular weight compounds, i.e. $^3$H inulin (MW 5000 daltons) and fluorescent dextran (MW 4000 daltons), were applied to the post auricular bald spot. It was considered that neither compound would penetrate the skin in significant amounts, and therefore any activity recovered in the receptor compartment would be as a direct result of contamination of the experimental system.

## METHODS

White, female Hartley guinea-pigs weighing 350–500 gm were housed individually in metabolic cages and fed standard guinea-pig chow and water *ad libitum*. Ambient temperature was 72 ± 2°F at 50–70% relative humidity.

### *Experiment 1*

$^3$H-labelled inulin was dissolved in methanol. 50 $\mu$l was applied to one post auricular site on each of six guinea-pigs. The animals were placed in individual metabolic cages and urine was collected over 6 h periods in a fraction collector for a total period of 48 h. Faeces were collected at 24 and 48 h. At 24 h, three animals were removed from the experiment. Each cage bottom was washed with 100 ml of 70% methanol and the liquid collected. The same procedure was adopted at 48 h for the remaining three animals.

### *Experiment 2*

Fluorescent dextran (aq) was applied to the post auricular site of six guinea-pigs. Each was housed individually in a metabolic cage. At the end of one 24-h period both the animals and the cages were examined by ultraviolet light.

### *Experiment 3*

$^3$H-labelled inulin in methanol was applied, under total occlusion, to the shaved backs of six guinea-pigs. Urine was collected over 24 h for four 6 h periods.

### *Experiment 4*

Excised, shaved, full-thickness pieces of guinea-pig skin were placed on six flow-through diffusion cells. 100 $\mu$l of $^3$H-labelled inulin in methanol was placed on the skin surface. Each cell was perfused continuously with a physiological saline receptor fluid at a rate of 5 ml per hour. Samples of the receptor fluid were collected continuously using a fraction collector over a 20 h period in 1 h units.

In Experiments 1, 3 and 4, the level of radioactivity ($^3$H) was measured by liquid scintillation spectroscopy. Duplicate vials containing internal standards were also measured to determine the extent of quenching.

## RESULTS

### *Experiment 1. $^3$H inulin*

On application of the inulin in a methanol vehicle the skin was obviously wetted. The methanol

TABLE 1. $^3$H activity recovered from the urine and the cage bottom expressed as percentage of the total dose applied

| Time (hours) | % of the total applied dose recovered in each time period (mean ± SD) | |
|---|---|---|
| | Urine | Cage bottom |
| 0–6 | 0·26 ± 0·008 $n=6$ | |
| 6–12 | 1·15 ± 0·82 $n=6$ | |
| 12–18 | 1·49 ± 0·58 $n=6$ | |
| 18–24 | 1·91 ± 2·71 $n=6$ | 12·75 ± 5·53 $n=6$ |
| 24–30 | 3·38 ± 3·00 $n=3$ | |
| 30–36 | 4·43 ± 2·90 $n=3$ | |
| 36–42 | 8·86 ± 8·87 $n=3$ | |
| 42–48 | 2·14 ± 2·00 $n=3$ | 17·80 ± 2·02 $n=3$ |

evaporated slowly to leave a white powder on the skin surface. Table 1 shows the mean amount of $^3$H activity recovered from the urine expressed as a percentage of the total dose applied (column 2). Column 3 (Table 1) shows the amount of $^3$H activity recovered from the cage bottom expressed as a percentage of the total dose applied.

*Experiment 2. Fluorescent dextran*

At the end of 24 h, examination of both the animals and the cages revealed the following: (i) intense green fluorescence at the site of application; (ii) slight spreading of the fluorescence onto the surrounding fur but less than 0·5 cm in any direction from the site of application; (iii) no obvious fluorescence on the paws or nasal/oral regions of the animals, and (iv) a remarkably uniform spread of fluorescent marker over the base of each metabolic cage. No fluorescence was found under or in the feeding trough. Fluorescence was also found in the delivery funnel beneath each cage.

TABLE 2. Mean $^3$H activity recovered from the urine expressed as a percentage of the total dose applied per 6 h period. $^3$H was applied under total occlusion

| Time (hours) | % of applied dose recovered in each time period |
|---|---|
| 0–6 | 0·004 $n=6$ |
| 6–12 | 0·001 $n=6$ |
| 12–18 | 0·01 $n=6$ |
| 18–24 | 0·06 $n=6$ |
| 24–36 | 0·04 $n=6$ |
| 36–42 | 0·01 $n=6$ |
| 42–48 | 0·08 $n=6$ |

*Experiment 3. $^3H$ inulin (total occlusion)*

Table 2 shows the mean amount of activity, expressed as a percentage of the total dose applied, recovered from the urine of each guinea-pig.

*Experiment 4. $^3H$ inulin* in vitro

The mean total amount of activity recovered in 20 h from the receptor phase was less than 0·01% of the total dose applied. The maximum recovered from the receptor 'compartment', i.e. the area below the skin and the receptor fluid collected in the fraction collector, of any one diffusion cell was 0·015% of the applied dose over 20 h.

## DISCUSSION

We have conducted a series of experiments which illustrates that caution must be exercised in the interpretation of *in vivo* percutaneous penetration data obtained from a typical rodent model.

Both inulin and dextran were chosen on the premise that compounds in excess of 1500 daltons molecular weight would probably not penetrate intact skin in significant amounts. Therefore, any activity measured in the receptor compartment of a metabolic cage would result from contamination of the system. The results shown in Table 1 indicate that, depending on where the guinea-pig urinates in the cage, there is a large amount of $^3$H activity that could appear in the collected urine, i.e. 7·66% at 24 h and at least 18% at 48 h. In this way, a curve constructed from radioactivity collected in the urine samples, plotted against time, would not give a reliable indication of either the amount penetrating or the profile of penetration.

It was considered that the large molecule may have been cleaved on the skin surface and a small molecule containing the $^3$H label actually penetrated into the animal. However, the obvious lack of penetration of inulin *in vitro*, and under occlusion *in vivo*, where one might expect enhanced penetration due to occlusion, implies that the molecule simply does not penetrate. The extremely low levels recovered *in vitro* at each 1 h time interval, i.e. < 0·007% of the applied dose, reflect scintillation counting levels only just above background, and may be due to either experimental error or the presence of the tritium label on a molecule other than inulin. Similarly, the authors consider that the levels of $^3$H activity recorded *in vitro* over 20 h (0·01%) and *in vivo* under occlusion at 18 h, and the subsequent 6 h periods, is not directly attributable to penetration of the inulin molecule.

In Experiment 2, fluorescent dextran gave a qualitative measurement of contamination of the experimental system. Although we could not conclude exactly how the animals managed to spread the fluorescent marker so evenly about the cage floor without obviously contaminating themselves, the experiment does serve to illustrate the unexpected wide distribution of the test molecule under certain experimental conditions. Again this indicates that, depending on where precisely and how much the guinea-pig urinates, the results from this style of experiment should be viewed with caution and should be substantiated by control experiments similar to those described. Simply covering or occluding the site of application is not sufficient as occlusion is known to enhance the penetration of several molecules. Covering the site may also change the relative availability of the molecule if it should show any affinity for the covering material. A number of devices are used to contain the dose in close proximity to the skin, e.g. Hill top chamber (Gummer & Maibach, 1985), with each worker having a preferred design and means of attachment. However, care should be taken to assess the degree of affinity of the molecule to, and its loss through, the covering and any changes in thermodynamic activity that may occur under conditions of partial or total occlusion. With such factors taken into account, published *in vitro* and *in vivo* penetration data for a particular molecule often shows good positive correlation (Bronaugh, 1985).

The authors accept that both compounds show relatively low solubilities and, once the vehicle component has evaporated, both compounds are deposited as visible solids on the skin surface. The results are therefore probably in exaggeration of data obtained from more typical drug molecules. However, in the presence of volatile vehicles, or where the test molecule is applied as a saturated solution, or where the test molecule is sparingly soluble and may be in suspension, care should be exercised in experimental design in order to avoid erroneous data.

Such experimental problems are obviously not confined to this particular animal model and should be considered in the design of any *in vivo* system where the subject is not totally immobilized.

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# Seborrhoeic eczema—a disease entity or a clinical variant of atopic eczema?

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## SUMMARY

One hundred and thirty-eight children seen at the Dermatology Clinic between 1969 and 1972 were reviewed. The study comprised two groups—a study group of 76 children diagnosed as having seborrhoeic eczema, and a group of 62 children seen over the same time period at the same clinic and diagnosed as having atopic eczema. The children were examined and a family history obtained with particular reference to past or present history of atopic diseases. Serum IgE, blood eosinophil count, pulmonary function tests and skin prick testing to various allergens were measured in the two groups. In the seborrhoeic group, 19% had eczema, 20% had abnormal pulmonary function clinically; 25% had a raised eosinophil count, 44% a raised IgE level, 89% positive skin prick testing, and 52% a peak flow less than 80% of predicted value. These results indicate a closer than expected association between infantile seborrhoeic eczema and atopic disease.

Infantile seborrhoeic eczema is conventionally considered by many to be a distinct disease entity separate from atopic disease (Yates, Kerr & Mackie, 1983a), while others hold the view that it is simply a clinical variant of atopic disease (Vickers, 1980). Prognostically, this is important not only dermatologically but also in terms of possible predisposition to other atopic manifestations such as asthma or allergic rhinitis.

In this study, a sequel to the review of children with infantile eczema reported by Burrows (1960), children diagnosed as having seborrhoeic eczema in infancy were assessed for evidence of atopy and respiratory symptoms 10–14 years after initial presentation, and the results were compared with a group of children diagnosed as having atopic eczema in infancy.

## METHODS

Ninety-two children aged from birth to one year who presented between 1969 and 1972 were diagnosed as having seborrhoeic eczema, based on the clinical criteria of Beare and Rook (1979).

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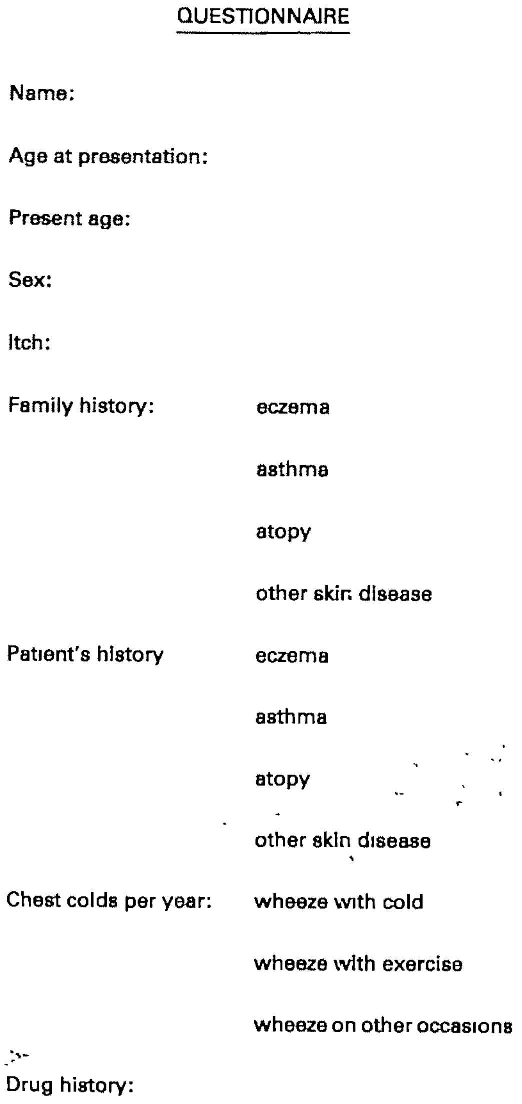
All had greasy skin involvement of scalp, napkin area and post-auricular areas, with early clearance and low incidence of itch. Seventy-six of these children were traced and formed the study group. Eighty children aged from birth to 2 years, who presented between 1969 and 1972, were diagnosed as having atopic eczema, based on the clinical criteria of Hanifin and Rajka (1980). All had an erythematous, often itchy, weeping papulo-vesicular eruption with either facial or generalized body involvement, but relative sparing of the scalp and napkin area in the very young babies, or flexural eczema in the older infants. Sixty-two of these were traced and formed the comparison group. All 138 children were interviewed with their parents, examined, and a questionnaire completed (Fig. 1). Asthma was diagnosed on clinical history and 'atopy' signified allergic rhinitis or history of penicillin allergy. Seventy-two of the seborrhoeic group



QUESTIONNAIRE

Name:

Age at presentation:

Present age:

Sex:

Itch:

| | |
|---|---|
| Family history: | eczema |
| | asthma |
| | atopy |
| | other skin disease |
| Patient's history | eczema |
| | asthma |
| | atopy |
| | other skin disease |
| Chest colds per year: | wheeze with cold |
| | wheeze with exercise |
| | wheeze on other occasions |

Drug history:

FIGURE 1. Questionnaire used in the study.

and 56 of the atopic group attended the dermatology department where blood eosinophil count, serum IgE levels and pulmonary function tests were measured.

Serum IgE was assayed using a modification of the sandwich technique (Kjellman, 1976a), the Tandem R radioimmunometric assay. The upper limit of normal for the study was accepted as 120 IU/l and the upper limit of normal for the eosinophil count was accepted as 500/$\mu$l.

Peak expiratory flow rate (PEFR), forced expiratory volume in one second ($FEV_1$) and forced vital capacity (FVC) were measured in both groups from flow volume curves and expressed as absolute values and as percentage of the predicted value corrected for age, sex and height.

Forty-two of the study group and 52 of the control group had skin prick testing to house dust mite, house dust, grass pollens, dog, cat and *Aspergillus fumigatus* antigens. A positive control (histamine) and a negative control (diluent) were recorded on each child. A positive result was taken as one in which the diameter of the test weal was greater than the diameter of the negative diluent control.

A representative age- and sex-matched group of 100 local school children was recruited and full personal and family history of past or present dermatological or atopic disease recorded to provide information on local prevalence of atopic disease.

Qualitative data were compared between the two groups using the chi-squared test. Quantitative data ($FEV_1$/FVC % predicted, eosinophil count, IgE and skin test results) were analysed using Student's *t*-test for normally distributed values, and the Mann-Whitney U test for data with a non-normal distribution.

## RESULTS

The age range of the 76 children in the seborrhoeic group was from 11 to 14 years, with a mean of 12·8 years and a male predominance of 71%. The mean age at onset was 4 months, with a range of 3 weeks to one year. Itch was recorded if a positive history was given by the parent, if scratch marks were noted on examination, or if antihistamines had been prescribed for itch. Only eight children (11%) had this symptom at presentation.

In the atopic group, children aged up to 2 years at presentation were included in order to give a comparison group of adequate size. The mean age at onset was therefore later, at 11·8 months, with a range at presentation of 4 months to 2 years. The age range at the time of follow-up examination was 12–17 years, with a mean of 14·6 years. Again, there was a male predominance of 65%. In this group, 58% had itch on presentation, and many of these children had itched intensely as infants.

Table 1 shows the percentage of children in the seborrhoeic, atopic eczema and control groups with atopy-associated problems at the time of interview. It can be seen that the seborrhoeic group are intermediate in the frequency of these problems, as compared with the other two groups, and that this atopic tendency extends to the families of the patients. On examination at review, atopic eczema was noted in 19% of the seborrhoeic group as compared with 10% of the control group and 45% of those originally presenting with atopic eczema. Atopic eczema was of the typical flexural pattern in all cases and most were regularly attending dermatology clinics and receiving either continuous or intermittent topical steroids.

Table 2 shows the other skin conditions observed in the seborrhoeic and atopic groups seen at the time of interview.

Table 3 shows that similar percentages in the two ezcema groups had frequent chest colds together with the classical exercise-induced asthma. Although the seborrhoeic group had slightly less wheeze at other times, a similar percentage overall had received anti-asthmatic

TABLE 1. Incidence of personal and family history of atopy in the three study groups

| | Seborrhoeic group | | Local school-children | | Atopic group | |
|---|---|---|---|---|---|---|
| | No. of patients | % | No. of patients | % | No. of patients | % |
| Current personal history: | | | | | | |
| Atopic eczema | 15 | 19 | 10 | 10 | 28 | 45 |
| Asthma | 13 | 17 | 9 | 9 | 21 | 35 |
| Atopy, other than eczema or asthma | 10 | 13 | 8 | 8 | 22 | 36 |
| Other skin disease | 21 | 28 | 6 | 6 | 6 | 10 |
| Family history: | | | | | | |
| Eczema | 18 | 23 | 7 | 7 | 25 | 40 |
| Asthma | 24 | 31 | 8 | 8 | 28 | 45 |
| Atopy, other than eczema or asthma | 8 | 10 | 5 | 5 | 10 | 16 |
| Other skin disease | 11 | 15 | 4 | 4 | 6 | 10 |

TABLE 2. Other skin diseases in seborrhoeic and atopic groups

| | Seborrhoeic group | Atopic group |
|---|---|---|
| Psoriasis | 5 | 1 |
| Seborrhoeic eczema | 6 | 1 |
| Icthyosis | 2 | 0 |
| Hand warts | 1 | 1 |
| Nickel allergy | 2 | 1 |
| Tinea | 1 | 0 |
| Acne | 3 | 2 |
| Giannotti Crosti syndrome | 1 | 0 |
| Total (%) | 21 (28) | 6 (10) |

drugs. As can been seen from Table 4, there is only a small statistical difference in the $FEV_1$/FVC ratio expressed as a percentage of predicted ($P = 0{\cdot}015$). However, 52% of the seborrhoeic group had a PEFR of less than 80% predicted, which is the level generally used to indicate a clinically significant abnormal value. We have used the PEFR for subsequent comparisons with other tests of atopy.

Table 5 compares the eosinophil counts and serum IgE levels. Only the mean values show statistically significant differences between the two eczema groups ($P < 0{\cdot}05$). Twenty-five per cent of the seborrhoeic group and 35% of the atopic group had abnormally high eosinophil counts while total serum IgE values were elevated in 44% of the seborrhoeic group and 55% of the atopic group.

The results of skin prick testing are shown in Table 6. The positive results were sub-divided

TABLE 3. Clinical respiratory status in seborrhoeic and atopic groups

| | Seborrhoeic group | | Atopic group | |
|---|---|---|---|---|
| | No. of patients | % | No. of patients | % |
| > 3 chest colds per year | 16 | 21 | 13 | 22 |
| Wheeze with colds | 14 | 18 | 21 | 33 |
| Wheeze with exercise | 18 | 23 | 17 | 27 |
| Wheeze at other times | 2 | 3 | 7 | 12 |
| Taking respiratory drugs at present | 8 | 10 | 11 | 18 |
| Past history of taking respiratory drugs | 8 | 10 | 3 | 5 |

TABLE 4. Pulmonary function tests

| | Seborrhoeic group | Atopic group |
|---|---|---|
| FVC % predicted | 91·0 ± 11·460 | 92·0 ± 11·460 |
| $FEV_1$ % predicted | 83·0 ± 13·080 | 81·0 ± 12·550 |
| $FEV_1$/FVC % predicted | 96·9 ± 10·312* | 92·0 ± 11·293* |
| PF % predicted | 80·0 ± 14·502 | 78·0 ± 12·641 |

Values are means ± SD.

* Statistically significant difference between the two groups ($P$ = 0·015; Students $t$-test).

TABLE 5. Eosinophil counts and IgE levels

| | Seborrhoeic group | | Atopic group | |
|---|---|---|---|---|
| | No. of patients | % | No. of patients | % |
| Eosinophil count (cells/ml) | | | | |
| < 500 | 38 | 53 | 24 | 42 |
| 500–700 | 16 | 22 | 13 | 23 |
| > 700 | 18 | 25 | 20 | 35 |
| Mean ± SD | 341 ± 316·45* | | 317 ± 248·70* | |
| IgE (IU/l) | | | | |
| < 120 | 38 | 56 | 25 | 45 |
| 120–400 | 13 | 19 | 12 | 22 |
| > 400 | 16 | 25 | 19 | 33 |
| Mean ± SD | 264 ± 331·79* | | 339 ± 324·89* | |

* $P < 0·05$, Mann Whitney U test.

TABLE 6. Numbers of patients with type I skin reactions

| Type I skin test diameter (mm) | Number of patients (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grass pollens | | House dust | | House dust mite | | Dog | | Cat | | *Aspergillus fumigatus* | |
| | A | S | A | S | A | S | A | S | A | S | A | S |
| Negative | 21(40) | 25(59) | 18(35) | 18(43) | 13(25) | 13(31) | 32(62) | 27(64) | 31(60) | 30(72) | 45(86) | 33(78) |
| 1–2 mm* | 4(8) | 14(33) | 5(10) | 21(50) | 6(12) | 22(52) | 9(17) | 15(36) | 6(12) | 11(26) | 3(6) | 8(20) |
| > 2 mm* | 27(52) | 3(8) | 29(55) | 3(7) | 33(63) | 7(17) | 11(21) | 0 | 15(28) | 1(2) | 4(8) | 1(2) |
| Mean weal size ± SD | 3·1**<br>±3·2 | 0·8<br>±1·39 | 2·5**<br>±2·2 | 1·07<br>±1·17 | 3·7**<br>±3·07 | 2·59<br>±2·44 | 0·99<br>±1·56 | 0·59<br>±0·85 | 1·35**<br>±1·92 | 0·50<br>±0·94 | 0·33<br>±0·97 | 0·35<br>±0·73 |

* Reaction diameter greater than that of control.
** $P < 0{\cdot}05$, Student's *t*-test.
A = Atopic group.
S = Seborrhoeic group.

into weakly positive (1–2 mm greater than the negative control) and strongly positive (more than 2 mm greater than the negative control). The mean weal size caused by each allergen, except for *Aspergillus fumigatus* and dog dander, was significantly greater for the atopic patients than the seborrhoeic patients ($P < 0{\cdot}05$). Although smaller in diameter than in the atopic group, the incidence of positive reactions was still very high in the seborrhoeic patients, with 89% giving a positive reaction to at least one of the antigens and 69% to house dust mite. Of those who had strong reactions, 64% suffered from asthma and 59% had a PEFR less than 80% predicted.

Table 7 demonstrates that those seborrhoeic patients with an eosinophilia also had higher serum IgE levels and airflow obstruction (PEFR < 80% predicted). Seventy per cent of those patients who had a raised serum IgE level also had airflow obstruction (Table 8).

TABLE 7. Correlation of eosinophil count with serum IgE level and peak expiratory flow rate (PEFR) in seborrhoeic group

| | Eosinophil count | |
|---|---|---|
| | < 500 cells/ml ($n$ = 52, 75%) | > 500 cells/ml ($n$ = 18, 25%) |
| IgE > 120 IU/l | 36% | 83% |
| IgE < 120 IU/l | 64% | 17% |
| PEFR < 80% predicted | 42% | 72% |
| PEFR > 80% predicted | 58% | 28% |

TABLE 8. Correlation of IgE level with the peak expiratory flow rate in the seborrhoeic group

| | IgE > 120 IU/l ($n$ = 32) | IgE < 120 IU/l ($n$ = 38) |
|---|---|---|
| PEFR > 80% | 30% | 70% |
| PEFR < 80% | 70% | 30% |

## DISCUSSION

At the start of this study, the classical clinical pattern of seborrhoeic dermatitis, i.e. early onset of yellow-red greasy, scaly rash with napkin area, scalp, post-auricular and skin crease involvement, with early clearance and low incidence of itch as described by Mauro and Lumpkin (1977), Beare and Rook (1979), Yates *et al.* (1983a) and Rystedt (1985) was accepted as a distinct disease entity. This was considered to be a separate condition from atopic eczema which classically, in young babies, is an erythematous, vesicular weeping rash of more general distribution, including the face, but with relative sparing of the napkin area and scalp. In older infants with atopic eczema a flexural pattern is seen. Atopic eczema tends to be of later onset with a higher incidence of itch and tends to be intractable. However, Vickers (1980) held the view that seborrhoeic eczema, like reversed pattern eczema, is simply a variant of atopic eczema with a distinctly different prognosis.

Neville and Finn (1975) sub-divided children with infantile seborrhoeic eczema into four patterns—psoriasiform, seborrhoeic, localized napkin dermatitis and generalized napkin dermatitis. On review 10 years later, 17% in the psoriasiform group had psoriasis; 37% in the seborrhoeic group had atopic eczema. The predicted incidence in both cases was 0·4%, suggesting a strong atopic tendency in those who developed seborrhoeic pattern infantile eczema.

If seborrhoeic eczema is clinically distinct from atopic eczema and atopic disease, then the incidence of personal and family atopic disease should correlate with the local incidence statistics as recorded in our group of 100 local school children. Although the atopic group had a statistically significant higher incidence of personal and family history of atopy using the chi-squared test, the figures for the seborrhoeic group were markedly higher than those for the normal school children. Local figures were determined as the prevalence of atopic eczema shows regional variation (Rystedt, 1985). Beare and Rook (1979) quoted a range of 2–20%. However, the figure for local prevalence of eczema of 10% corresponds closely to that of 12·2% quoted by Taylor *et al.* (1984) for children born in 1970, in their nationwide epidemiological survey of prevalence of ezcema.

Forty-four per cent of the seborrhoeic group had an elevated IgE level which was substantial in 25%. Statistically, there was no significant difference between the two groups, but the general trend was for higher levels of IgE in the atopic group. Kjellman (1976a) found that in 186 children selected out of 226 on the grounds of no personal or family history of atopic disease, no child had a serum IgE level above 100 IU/l. Others have shown a significant correlation between raised IgE levels and the presence of or subsequent development of atopy (Turner, Rothman & O'Mahoney, 1974; Orgel *et al.*, 1975; Kjellman, 1976b; Yates *et al.*, 1983b).

In a follow-up study of 955 patients who had had atopic eczema in childhood, Rystedt (1985) found that a raised IgE level was associated with past or present symptoms of respiratory allergy in all but 17. She also noted a strong association between IgE levels and the extent of persistent dermatitis.

Isolated eosinophil counts are not of great value in that the eosinophil level may vary even from day to day in any individual (Yates *et al.*, 1983b). However, as this was a retrospective study, it was difficult to justify more than one visit to the hospital. We were, however, able to show that not only a high percentage of the seborrhoeic group had eosinophilia but also that there was a strong correlation with raised serum IgE levels and depressed bronchial airflow.

Respiratory status was assessed both clinically and with measured pulmonary function tests corrected for age, sex and height by using the percentage predicted values and, thus, allowing comparison of the slightly older atopic to the seborrhoeic group. Asthmatic children on the average tend to have three chest colds per annum, compared to less than two in normal children, and asthmatic children may wheeze with upper respiratory tract infections and with exercise, a finding less likely in normal children (Orgel, 1975). The historical parameters are a useful indicator since, even in asthmatic children, pulmonary function tests may be normal between bouts of asthma. A PEFR $<80\%$ predicted is generally accepted as abnormal. Fifty-two per cent of the seborrhoeic group fell below this level and although the atopic group had more airflow obstruction, there was only a small difference between the two groups.

Skin prick testing is a useful, inexpensive, sensitive, safe and specific method for identifying allergy (Canadian Pediatric Society, 1983). In this study, 89% of the seborrhoeic group had a positive skin test, similar to the figure of 86% quoted by Hendrick (1975) in his group of 656 asthmatic patients who had skin prick testing carried out. Figures quoted for the normal population vary from study to study. Godfrey and Griffiths (1976) reported 34% positive results to house dust mite and grass pollen in a randomly selected group of British children. Excluding children with atopic manifestations, this figure fell to 20% and ranges from 7% to 15% have been reported in other studies (Williams & McNichol, 1969). In both the atopic and the seborrhoeic group the most commonly positive result was to house dust mite, followed by house dust, grass pollens, animal danders and aspergillus. For all of the allergens, the atopic group were more strongly positive than the seborrhoeic group.

Itch is considered by some to be helpful diagnostically (Rystedt, 1985). Of the seborrhoeic group, 11% were recorded as having itch compared to 58% of the atopic group. Despite the retrospective nature of this study, these figures would indicate that itch is useful in differentiating these two patterns of eczema. Only half of the seborrhoeic group with itch were skin tested but they all showed positive results.

We demonstrated that patients diagnosed as having seborrhoeic eczema in infancy subsequently have an increased incidence of atopy which is intermediate between the incidence in normal controls and atopic patients. Correlations between studied atopic phenomena were of a significant strength to suggest that seborrhoeic eczema in infancy is another variant of the atopic tendency. McNichol and Williams (1973) observed that a cluster of atopic parameters was the differentiating feature of atopic disease but that no one atopic marker identified atopics unequivocally. In our study group there was a high concurrence of markers of atopy.

The findings suggest that, although seborrhoeic eczema and atopic eczema may be distinguished clinically (Beare & Rook, 1979), children with seborrhoeic eczema in infancy have a higher than normal incidence of atopic manifestations in later childhood and thus seborrhoeic eczema may be part of the spectrum of atopic disease with resultant prognostic implications.

## ACKNOWLEDGMENTS

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# Immunogold labelling of epidermal Langerhans cells on tissue sections of normal human skin

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## SUMMARY

A colloidal gold staining procedure was developed for immuno-electron microscopy, which allowed the demonstration of T6 surface antigens of epidermal Langerhans cells on sections of normal human skin with consistent ultrastructural preservation of subcellular structures. All the observed Birbeck granule-bearing Langerhans cells revealed immunogold labelling along the plasma membrane. The penetration of the tissue by the gold particles was adequate, as was the sensitivity and the specificity of the reaction. A major advantage of the present method in comparison with immunoperoxidase techniques is the absence of non-specific staining of the membranes of keratinocytes facing the labelled plasma membrane of the Langerhans cells.

Monoclonal antibodies (MA), when tagged with fluorescein, enzymes or other appropriate labels, are used successfully for specific *in situ* demonstrations of cell-surface antigens of epidermis and dermis at the light microscopic level. For cutaneous immuno-electron microscopy (IEM), MA have to be tagged with an electron-dense marker. Immunocolloidal gold (IG) methods have several important advantages in comparison with other IEM techniques, such as immunoperoxidase (IP) (Novikoff, 1980; Courtoy, Picton & Farquhar, 1983) and immunoferritin (Roth, Bendayan & Orci, 1978; Evans & Webb, 1984). Colloidal gold is particulate, thus allowing accurate identification of the labelled structures, double labelling and quantitative evaluation of the intensity of the labelling (Bendayan, 1984).

Langerhans cells (LC) are well known as important cells responsible for the immune functions of the skin (Stingl *et al.*, 1978). Together with a distinctive cytoplasmic organelle, the Birbeck granule, LC express cytoplasmic and surface markers, which are used to distinguish them from resident and infiltrating epidermal cells (EC) (De Panfilis & Rowden, 1986). LC surface markers include the HLA-DR surface antigen and the surface antigen detected by OKT6 and related MA (T6). T6 positivity seems to be more sensitive by comparison with HLA-DR positivity on skin sections, as T6 positive cells are more numerous than HLA-DR

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positive cells as evaluated on *in situ* unextracted EC samples by the majority of authors (MacKie & Turbitt, 1983; De Panfilis, 1983; Harrist *et al.*, 1983; Haftek *et al.*, 1983).

Although particulate labels such as ferritin were originally used to demonstrate the specific reactivity of HLA-DR antigens of LC in IEM (Rowden, 1977), the detection of LC surface antigens by IG techniques has not yet been reported on skin sections, but only on EC suspensions. On skin sections, LC surface antigens are commonly detected in IEM by means of *in situ* IP methods (Holden, Morgan & MacDonald, 1982; Berti *et al.*, 1985; De Vos *et al.*, 1985). However, IG labelling of LC surface antigens is more sensitive than IP labelling (Schmitt *et al.*, 1984). Moreover, IP labelling shows poor specificity on skin sections, since reaction products diffuse from LC membranes to the membrane of facing EC (De Vos *et al.*, 1985).

In the present investigation IG labelling of LC surface antigens on sections of normal skin was established.

## METHODS

Five healthy volunteers provided normal human skin. Punch biopsies were taken from skin of the forearms.

### *Monoclonal antibodies and sera*

As MA detecting LC T6 surface antigens, OKT6 (Ortho, Raritan, NJ, U.S.A.) and anti-Leu-6 (Becton Dickinson, Mountain View, CA, U.S.A.) were used, diluted 1:10 in PBS. GAM G5 (Janssen, Beerse, Belgium), diluted 1:10 in 0·5 M tris buffer containing 1% bovine serum albumin (BSA; Sigma, St. Louis, MO, U.S.A.), was used as IG (5-nm sized) labelled goat antimouse serum.

As control sera, non-immune mouse ascites (Cappel, Plochingen, F.R.G.), and MA unreactive with LC (OKT3, OKT8, Ortho; anti-Leu-2a, anti-Leu-4, Becton Dickinson) were used, all diluted 1:10 in PBS.

### *In situ immunogold labelling in immunoelectronmicroscopy*

Skin specimens were prefixed first in 4% paraformaldehyde for 10 min, washed in PBS, and incubated in dimethylsulfoxide for 1 h. 10–20 μm thick cryostat sections were fixed 2 h in 4% paraformaldehyde, incubated for 12 h with MA, and, after 24 h washing in PBS, incubated for 12 h with the gold-labelled serum. After washing for 24 h in PBS containing 0·1% BSA, sections were fixed in 1% glutaraldehyde and treated routinely for the electron microscopy procedures, including postfixation in osmium, dehydration in acetone, embedding in Durcupan ACM, sectioning by a LKB III ultramicrotome, and examined by a Zeiss EM 109 electron microscope.

### *Controls*

For IG controls, ascites fluid replaced the MA in the first layer, or the first layer was omitted.

## RESULTS

Ultrastructural preservation of tissue sections of normal human epidermis was acceptable in the present IG *in situ* IEM procedure (Figs. 1a and 2a).

LC were first identified by the cytoplasmic evidence of Birbeck granules (Figs 1a and 2b). The Birbeck granule-bearing LC were detectable by the specific T6 IG surface labelling (Figs 1b and 2c).

The IG cell-surface labelling showed the presence of variable numbers of gold particles,

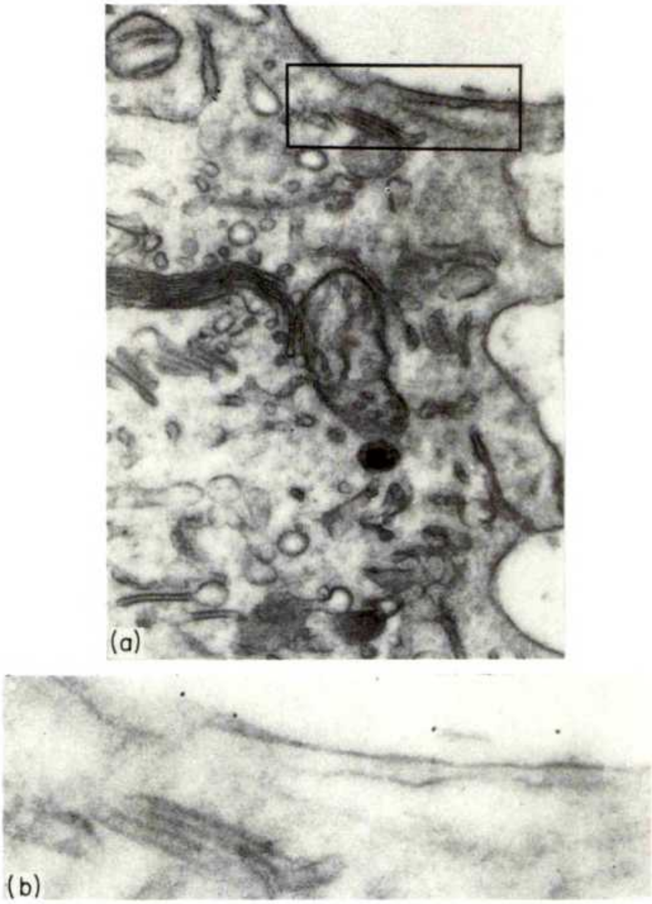

FIGURE 1. Section of normal human skin. An epidermal Langerhans cell is immunogold labelled (anti-Leu-6 monoclonal antibody). (a) Ultrastructural details of the cytoplasm, including Birbeck granules (original × 41825); (b) higher magnification in the cell surface of (a), showing specific T6 positive labelling, since gold particles are scattered along the plasma membrane (original × 112 500).

scattered along the LC plasma membrane, apposed to the cell surface (Figs 1b and 2c). No gold particles were visible in any intracellular location.

The cell surface of melanocytes and keratinocytes was always gold-negative. In particular, keratinocytes adjacent to IG labelled LC were not IG labelled (Fig. 2a), showing no evidence of gold granules on the cell membrane facing the gold-positive LC cell membrane (Fig 2c). LC were not labelled either when MA unreactive with LC were used, or in IG controls.

## DISCUSSION

The present investigation demonstrates that the IG labelling in IEM of surface antigens of LC, together with good ultrastructural preservation, is possible even on tissue sections. The LC IG labelling was highly sensitive, since all Birbeck granule-bearing cells seen were IG labelled. The

LC IG labelling was highly specific, since no gold particles were observed on the plasma membrane of EC facing the labelled LC plasma membrane.

The present *in situ* IG labelling of LC on skin sections seems to represent a genuine improvement in the specificity of IEM *in situ* techniques, as compared with IP labelling of LC on skin sections. In fact, even in recommended IP-IEM *in situ* procedures, peroxidase positivity on cell membranes of keratinocytes facing stained LC was noticed (de Vos *et al.*, 1985). Furthermore, in all sections of human epidermal sheets examined, peroxidase staining of the membranes of epithelial cells, where these membranes were adjacent to peroxidase-stained LC,

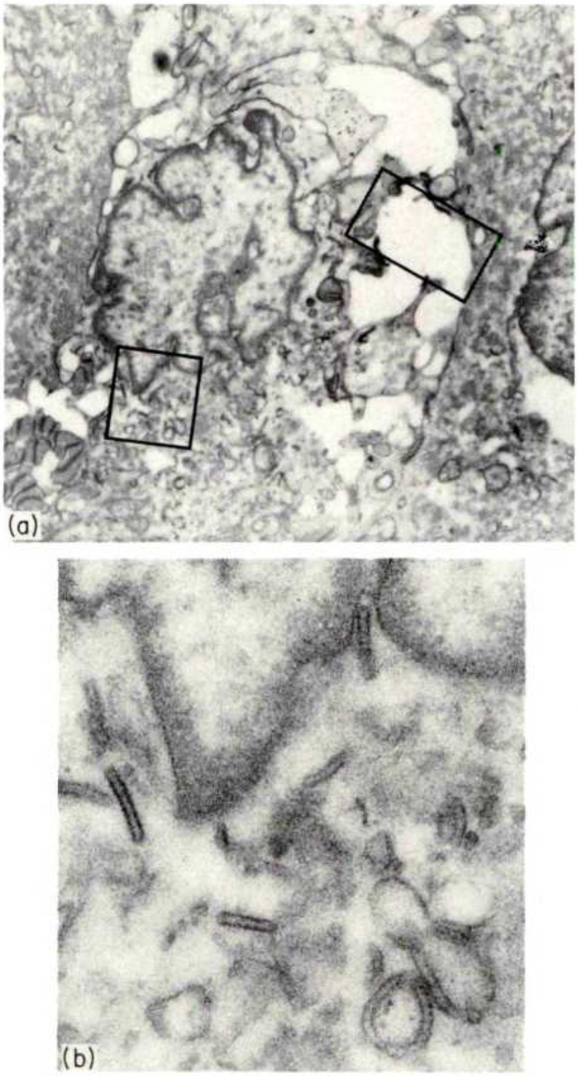

FIGURE 2. Section of normal human skin. An epidermal Langerhans cell is immunogold labelled (OKT6 monoclonal antibody). (a) Some keratinocytes are visible around the Langerhans cell (original × 7000); (b) in this detail of (a) some Birbeck granules are evident within the cytoplasm of the Langerhans cell (original × 65 000).

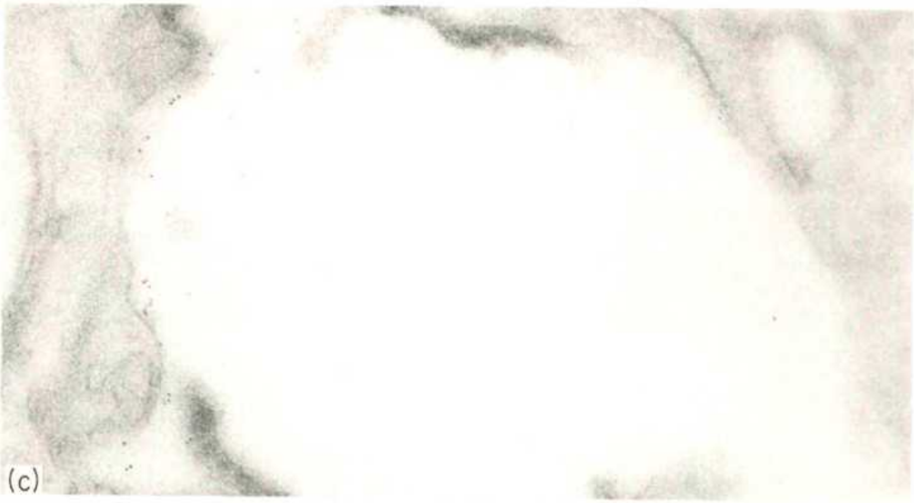

FIGURE 2. (c) In this detail of (a) the Langerhans cell (left) shows its T6 positivity, since gold particles are scattered along the cell surface, whilst no gold granules are visible on the surface of the facing keratinocyte (right) (original × 80 000).

was observed (Greveson, Robertson & Everall, 1982). Even in abnormal dermis, diffusion of the peroxidase reaction products produces peroxidase staining of a portion of the plasma membrane of lymphocytes in contact with peroxidase-positive LC (Berti *et al.*, 1985). The present study demonstrates that when IG, other than IP, IEM method is used on skin sections, no gold positivity is noted in keratinocytes facing gold-stained LC. Presumably, therefore, the peroxidase staining of the membrane of keratinocytes adjacent to positive LC is an artifact, rather than representing some function of the LC such as antigen transfer or a regulatory role in keratinization, as discussed by Greveson *et al.* (1982). On the other hand, the possibility of the non-specific diffusion of peroxidase reaction products has been demonstrated not only in skin sections but also in various different systems (Novikoff, 1980; Courtoy *et al.*, 1983; Messing, Stieber & Gonatas, 1985).

The 5 nm gold particle-conjugated antibody showed good penetration into 10–20 μm thick skin sections and did not require permeabilization procedures. 5 nm gold particles are well suited to reveal membrane-associated antigens, since the binding of gold granules to the cell surface appears to be determined primarily by the size of the particle. Small particles should be used when high-resolution labelling, such as membrane proteins, is required (Bendayan, 1984).

Quantitation of LC surface antigens, presumably related to the number of gold particles detectable on the LC plasma membrane (Dezutter-Dambuyant *et al.*, 1984), is now possible.

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## *Case Reports*

# Fixed drug eruption due to paracetamol

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### SUMMARY

A patient who developed a fixed drug eruption due to paracetamol is reported. There is only one previous account of this eruption occurring as a consequence of paracetamol therapy.

There is only one report in the literature of paracetamol causing a fixed drug eruption (Wilson, 1975), although a preparation of paracetamol compounded with chlormezanone was suspected as the cause in one other report (Savin, 1970). We report a man who developed a fixed drug eruption undoubtedly caused by paracetamol.

### CASE REPORT

The patient was a stoical 74-year-old man who had always disliked taking any medicines. On account of lower abdominal pain he was prescribed Panadol® (paracetamol 500 mg); 12 h after taking one tablet, he developed several tender areas on the skin of his face which faded over a few days; as far as he could recall, he did not think he had taken paracetamol tablets previously. He took no further medicines until 2 months later when, on account of a further episode of abdominal discomfort, he took another single paracetamol tablet and 12 h later the tender areas recurred on his face at the same (or similar) sites as before, and again faded over a few days. The patient was admitted to hospital subsequently for investigation of his abdominal pain; despite stating that he was 'allergic' to paracetamol, the patient was prescribed Distalgaesic® (paracetamol 325 mg and dextropropoxyphene hydrochloride 32·5 mg) for the pain, thereby effectively providing a 'blind' challenge of paracetamol. The day after taking one of these tablets he again developed tender areas over his face in what he thought were similar sites as previously; examination at the time revealed 15 well demarcated, slightly tender, violaceous plaques of 1–2 cm diameter on his forehead, chin, upper lips and cheeks (Figs 1 and 2), the appearance being characteristic of a fixed drug eruption. The lesions faded over several days.

The patient took no other drugs. In particular, his abdominal pain was not associated with constipation and he did not take laxatives in any form.

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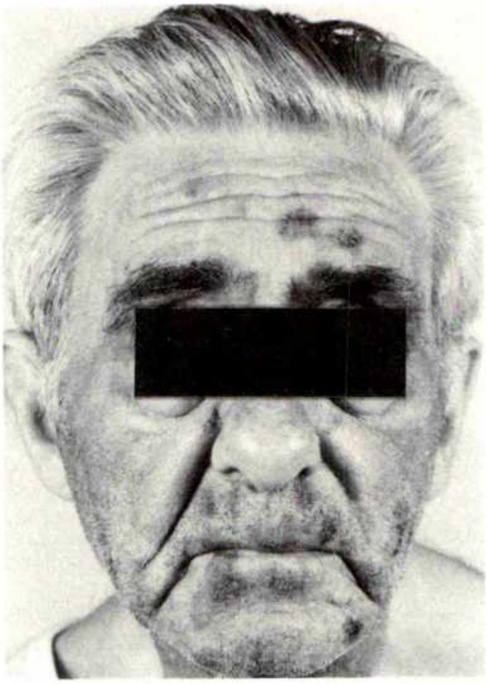

FIGURE 1. Lesions present on the face 48 h after ingestion of paracetamol 325 mg (in the form of one Distalgaesic tablet).

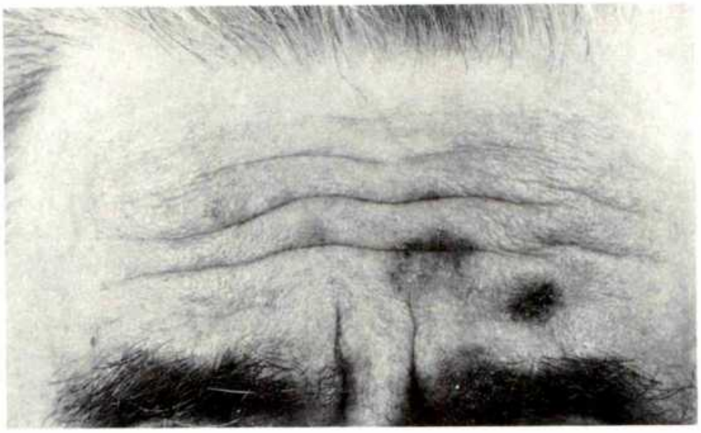

FIGURE 2. Close-up of lesions on the forehead.

Patch tests with 1% and 5% paracetamol in petrolatum were negative at 48 and 96 h; prick tests with 1% and 5% paracetamol in aqueous solution were attempted; however, the patient was found to be mildly dermographic and meaningful results could not, therefore, be obtained.

## DISCUSSION

In the 20 years between January 1964 and February 1984, the Committee on the Safety of Medicines (CSM) received a total of 396 reports of adverse reactions to paracetamol (including compounded preparations containing paracetamol), 129 of which were of skin reactions. As the specific cutaneous diagnosis of fixed drug reaction is not included by the CSM as a category for yellow card adverse drug reaction reports, it is not known how many—if any—of the skin reactions reported represented fixed drug eruption (CSM, personal communication).

Paracetamol is an extremely commonly used drug in the U.K. In 1983 (the most recent year for which accurate figures are available), an estimated 18 298 900 prescriptions for any preparation containing paracetamol were dispensed in the U.K. (DHSS Statistics and Research Division). It is likely that this figure underestimates considerably the actual usage of paracetamol, as the drug is also available without prescription. The number of paracetamol tablets consumed in Great Britain each year is not recorded; however, the manufacturers of the greatest quantity of the raw paracetamol drug estimate that 1400 million paracetamol tablets (excluding compounded preparations) are consumed annually in this country (Dr C.G. Nicol, personal communication).

Despite the drug's very widespread usage, fixed drug eruption must be an extremely uncommon reaction to paracetamol. In the major dermatological reference textbooks, paracetamol is not listed among drugs usually found to account for fixed drug eruption (Rook, Ebling & Wilkinson, 1979; Fitzpatrick *et al.*, 1979). It is possible, therefore, that paracetamol may be discounted inappropriately in the assessment of a patient presenting with a fixed drug eruption. Furthermore, some patients do not consider medicines available without prescription as 'drugs' and consequently may not mention paracetamol when asked to name any drugs they have taken. The cause of a fixed drug eruption is not always elucidated—in 44 cases assessed, the cause was not known in seven patients (16%) (Savin, 1979)—and it may be that a common self medication (such as paracetamol) occasionally accounts for such cases where the responsible drug remains elusive.

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# Periungual and subungual arteriovenous tumours

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## SUMMARY

We describe the clinicopathological findings in five cases of an acquired vascular tumour involving periungual or subungual tissue. We believe that these represent acral arteriovenous tumours (cirsoid aneurysms). There are no previous reports of subungual or periungual acral arteriovenous tumours.

## CASE REPORTS (TABLE 1)

### *Case 1*

A 55-year-old Caucasian female had a 4-year history of a painless nodule on the left little finger. There was no history of trauma. She had a firm, bluish, non-pulsatile vascular nodule in the lateral nail fold of the left little finger (Fig. 1).

The nodule was excised. On histological examination, there was a collection of abnormal small vessels of varying calibre in the dermis. The majority of vessels had thick fibrous walls containing no muscle (Fig. 2). There was no internal elastic lamina. The findings were consistent with a cirsoid aneurysm.

### *Case 2*

A 62-year-old Caucasian male presented with a 2-year history of painful lesions beneath the right thumb and index finger nails. The free edge of the nail plate had become notched. There was no history of trauma and he was not a manual worker. There were bluish, slightly tender lesions beneath the lunules of thumb and index finger nails. A red line extended from the lesions distally to the free nail border, where the nail plate was fissured and fragile (Fig. 3). There was no arthropathy; an X-ray was normal. A longitudinal nail biopsy was taken.

The proximal one-third of the specimen contained several small thick-walled vessels of varying calibre. Chronic granulation tissue occupied the distal two-thirds. There were no glomus cells. An angiomatous process, resembling a cirsoid aneurysm, was diagnosed.

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TABLE 1. Clinical details of lesions reported

| Case no. | Sex | Age (years) | Duration (years) | Site of lesion | Features |
|---|---|---|---|---|---|
| 1 | Female | 55 | 4 | Solitary periungual lesion | Painless bluish nodule |
| 2 | Male | 62 | 2 | Two subungual lesions | Bluish lunule, longitudinal red line; distal fissure, and fragility with splinter haemorrhages |
| 3 | Female | 53 | 1 | Two subungual lesions | Red lunule, longitudinal red line; distal fissure and fragility |
| 4 | Male | 48 | 6 | Solitary subungual lesion | Reddish lesion beneath nail plate; longitudinal red line; distal fissure and fragility with splinter haemorrhages |
| 5 | Male | 45 | 2 | Solitary subungual lesion | Reddish longitudinal ridge; distal fissure and fragility |

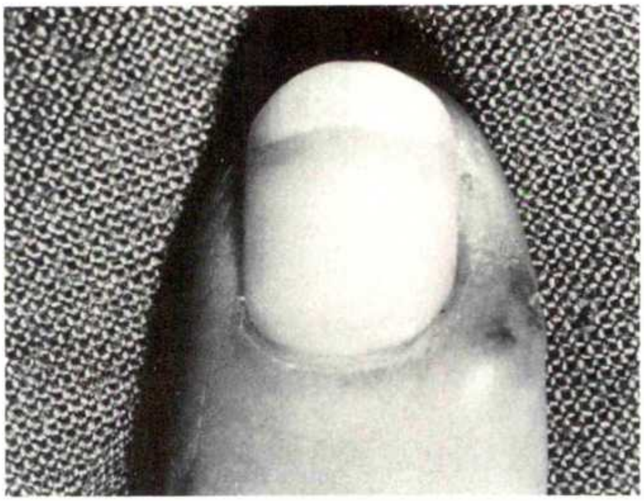

FIGURE 1. A firm blue nodule in the lateral nail fold of the left little finger.

*Case 3*

A 53-year-old Caucasian female had a 1-year history of tender thumb nails. The nails were not spontaneously painful and there was no history of trauma. The signs were similar to those in

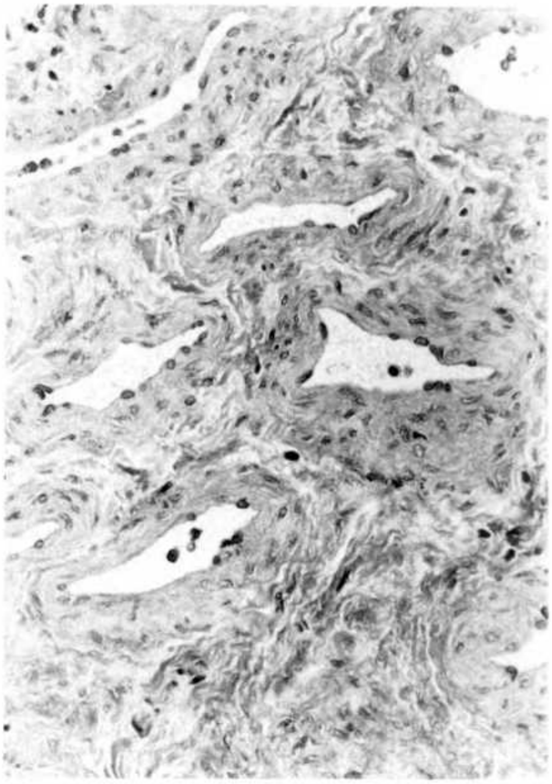

FIGURE 2. Thick-walled vascular channels. The walls are composed of fibrous tissue. There was no internal elastic lamina. (Massons trichrome stain; original × 440).

Case 2 (Fig. 3). The lunula was red, and a red line extended distally to the free edge of the nail, which was fragile.

A biopsy was taken from the nail matrix beneath the lunula. There was a localized dermal collection of thick-walled vessels (Fig. 4), associated with thinning of the overlying nail plate. The vascular walls were mainly fibrous.

### *Case 4*

A 48-year-old Caucasian male had had a painful lesion beneath the nail of the left middle finger for 6 years. There was no history of trauma. There was a localized moderately tender, reddish lesion beneath the nail plate. A longitudinal red line extended distally to the free nail border where there were a fissure and splinter haemorrhages. An X-ray was normal.

The nail plate was avulsed. There was a firm, 2 mm diameter pinkish nodule in the nail bed. This was removed with a 3 mm punch biopsy extending down to bone. The histology demonstrated prominent small veins and capillaries without glomus cells. The nail returned to normal.

### *Case 5*

A 45-year-old Caucasian male gave a 2-year history of nail tenderness. There was a reddish

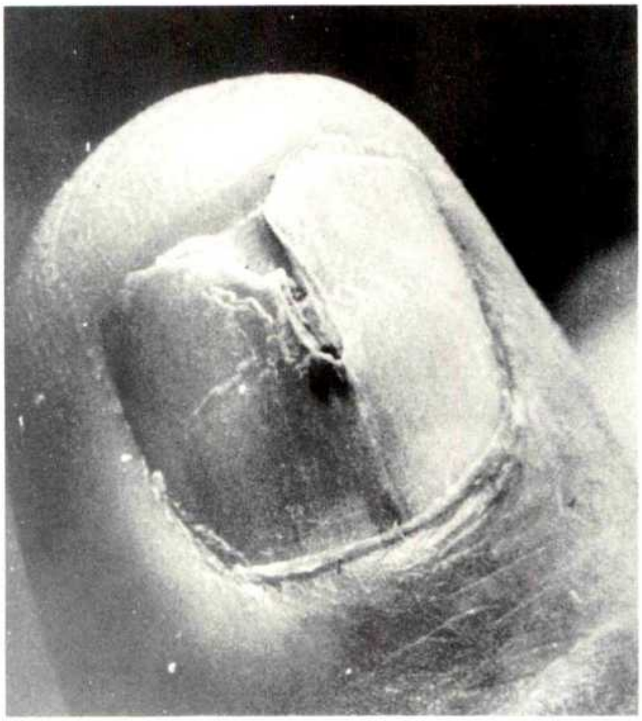

FIGURE 3. A bluish lesion beneath the lunule of the right thumb nail with a red line extending distally to a fissure.

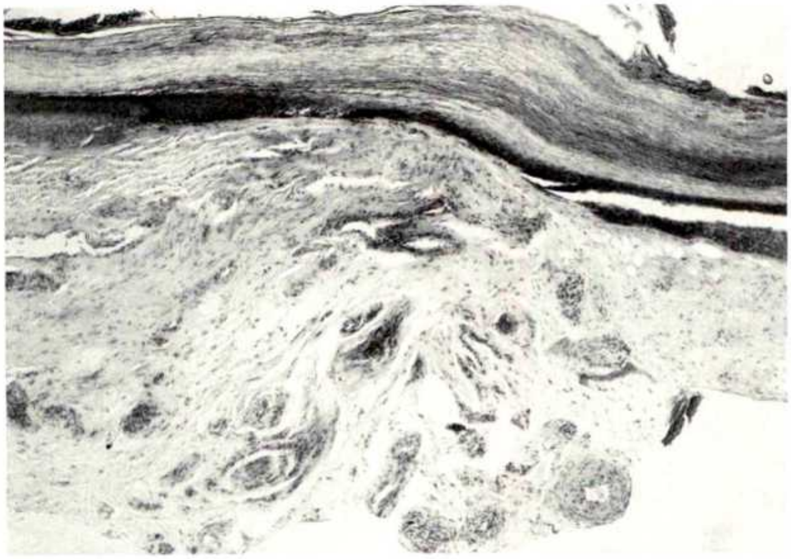

FIGURE 4. Superficial inflammation and a collection of thick-walled vessels of varying calibre in the subcutaneous tissue beneath the nail matrix. (H & E; original × 80).

longitudinal ridge extending from the edge of the lunula and ending in a distal fissure of the free edge of the nail. The nail plate was removed. There was a vascular tumour, less than 1 mm in diameter, visible using magnification, at the junction of nail matrix and nail bed. This was removed and the nail returned to normal. The histology merely showed prominent small vessels.

## DISCUSSION

The arteriovenous tumour, sometimes called a cirsoid aneurysm, which was described in the skin in 1956 (Biberstein & Jessner, 1956), has been the subject of two recent reviews (Carapeto, Garcia-Perez & Winkelmann, 1977; Connelly & Winkelmann, 1985). Although the tumour occurs predominantly in an acral distribution, only one of a series of 15 cases involved the dorsum of a finger (Carapeto *et al.*, 1977), and there have been no previous reports of subungual or periungual lesions.

Acral arteriovenous tumours are composed of thick-walled fibromuscular channels, which vary in diameter, and there is frequently an overlying capillary angioma component. Some authors have shown that there are direct communications between arteries and veins in the lesions (Girard, Graham & Johnson, 1974), and Connelly and Winkelmann (1985) agreed that the channels are probably transitional vessels which link arteries to veins. The histogenesis is uncertain, but the acral distribution is striking. Carapeto *et al.* (1977) were impressed by the histological similarities to the Sucquet-Hoyer canal of the glomus body and suggested that the lesions represented a hamartomatous proliferation of that structure. The lack of glomus cells and absence of a nerve component in the lesions are against this hypothesis, while the acral distribution provides some support. Glomus bodies are also found in large numbers beneath the nail and therefore periungual or subungal tumours would be expected.

The clinical and pathological features of our first three cases were consistent with a diagnosis of acral arteriovenous tumour (cirsoid aneurysm), but although the clinical features in the remaining two cases were similar, there was no histological confirmation of a tumour. The biopsies may have shown the overlying angiomatous capillary component which is common in these tumours, or alternatively the tumour was at an earlier stage with less well developed pathology. The interpretation of vascular changes in the nail bed is difficult, because this area is biopsied infrequently.

There was no history of trauma and the patients were all professional rather than manual workers. Therefore, we do not believe that trauma initiated these malformations, although we accept that trauma contributed to the changes in the free edge of the nail. The reddish line and fissuring resolved when the tumour was removed.

Subungual and periungual vascular tumours are not uncommon. Glomus tumours, pyogenic granulomata and developmental angiomatous naevi can involve the nail bed (Haneke, Baran & Bureau, 1984). Cirsoid aneurysm is distinguished from pyogenic granuloma by the prolonged history without enlargement or bleeding, while the absence of exquisite tenderness differentiates it from a glomus tumour.

In conclusion, we have described the features in one periungual and four subungual vascular tumours. We believe that these represent stages in the development of acral arteriovenous tumours (cirsoid aneurysm). A subungual vascular tumour should be considered in the differential diagnosis of causes of a longitudinal red line with distal fissuring in the nail.

## ACKNOWLEDGMENT

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# Thalidomide in the treatment of neuro-Behçet's syndrome

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## SUMMARY

No relapses of symptoms in a patient with neuro-Behçet's syndrome were observed after the addition of thalidomide to chlorambucil and prednisolone therapy. At present, the patient has been free of symptoms for 3 years. This observation, together with those reported from the literature, strongly support a re-evaluation of thalidomide therapy in certain life-threatening diseases such as neuro-Behçet's syndrome.

Behçet's syndrome is a disease entity with cutaneous, ocular, intestinal, articular, vascular and neurological manifestations (Alema, 1978). Lehner (1977) divided Behçet's syndrome into muco-cutaneous, arthritic, neurological and ocular varieties. The latter two carry a more serious prognosis. The neurological variety has been sub-classified as neuro-Behçet's syndrome (Cavaro & D'Ermo, 1954), which is commonly a lethal disease.

Definitive therapy for neuro-Behçet's syndrome does not exist, although the use of both corticosteroids and immunosuppressive therapy is generally advocated. We report a beneficial effect of thalidomide in a patient with neuro-Behçet's syndrome, who did not respond to conventional treatment with corticosteroids and chlorambucil.

## CASE REPORT

A 21-year-old Caucasian man was seen in January 1982 with multiple painful scrotal ulcerations which had been present for 3 weeks. He had a 3-year history of recurrent oral aphthosis. Penicillin sensitive, *S. aureus*, had been isolated from the scrotal lesions. The lesions were interpreted as impetigo and treated unsuccessfully with a sulphur paste and oral penicillin. The patient denied fever, weight loss, abdominal pain or arthralgia.

There was no inguinal adenopathy. Acneiform papules and pustules in a discrete pattern were

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observed over the face, shoulders and back. There was no hyper-irritability of the skin on the skin prick test.

Laboratory studies were as follows: ESR 8 mm/h, haemoglobin 8·3 mmol/l, WBC 8900/mm$^3$ with a normal differentiation. Liver and renal function tests were normal. Complement studies revealed normal levels of C3, C1q and CH50. Tests for circulating immune complexes were negative, as were the C1q binding assay and the indirect granulocyte phagocytosis test. Cryoglobulins were absent and analysis of peripheral lymphocytes showed normal percentages of B cells, T cells and T-lymphocyte subsets. Serological tests for syphilis and viral culture for herpes virus hominus were negative. Urine analysis showed no abnormalities.

A skin biopsy from the genital ulcers revealed a non-specific perivascular inflammatory reaction. The skin vessels showed some degree of dilatation, but there were no signs of vasculitis. Immunofluorescence showed focal granular deposits of IgM, C1q and C3 at the epidermal basement zone. The diagnosis Behçet's disease of an incomplete type (Inaba & Aoyama, 1982) was made. However, further examination revealed no ocular, pulmonary, vascular or intestinal abnormalities, nor involvement of the central nervous system. A complete remission of the genital ulcers was observed after treatment with 15 mg of prednisone daily for one week. Discontinuation of the therapy was followed by recurrent scrotal ulcers and oral aphthosis, varying in severity.

A few months later, the patient developed a fever up to 40°C and an inflammation of the right scrotal area, which was extremely painful on palpation. A diagnosis of epididymitis was made. After treatment with tetracycline these symptoms subsided.

Preceeded by episodes of severe frontal headache and fever up to 40°C, the patient was admitted to the Department of Neurology in March 1983 for a left-sided hemiparesis which had developed within a few days, with hyper-reflexia of tendon reflexes and a pathological plantar reflex on the left side. Electroencephalogram (EEG) showed an asymmetrical picture with delta waves in the right temporo-parieto-occipital area and involvement of deep structures. CT-scanning of the brain showed a hypodense pathological area in the posterior part of the right internal capsule with enhancement after contrast and surrounded by a small zone of oedema. Cerebro-spinal fluid (CSF) showed a mild pleocytosis (150/3 white cells-agranulocytes) and a slightly increased amount of protein (0·65 gc/h). A short period of psychiatric disorder occurred, in the form of an episode of an organic psychosyndrome consisting of confusion, disorientation and agitation.

The diagnosis of neuro-Behçet's syndrome was made and the patient was instituted on immunosuppressive treatment of chlorambucil 10 mg and prednisone 100 mg, both daily. Fever and headache subsided rapidly, together with the neurological and psychiatric symptoms, which disappeared almost completely except for a persisting hyper-reflexia on the left side. However, relapses of symptoms occurred while gradually reducing the doses of prednisone to 20 mg daily, and recurred again on a second trial with a prednisone dose of 30 mg daily. The patient complained of headache, fever, oral aphthosis and a black spot in the field of vision of his left eye, which appeared to be due to a temporal retinitis. Thalidomide 400 mg was added to the medication. Since then there have been no relapses, the EEG and CSF became normal and the CT scan showed only a persisting small area of hypodensity in the right internal capsule.

Since starting thalidomide 3 years ago, the patient has been completely free of symptoms on a maintenance dose of chlorambucil 2·5 mg daily, thalidomide 25 mg daily and prednisone 5 mg every other day. To date, no complications of the thalidomide medication, e.g. polyneuropathy, have been observed.

## DISCUSSION

The beneficial effect of thalidomide in the treatment of severe aphthous ulcers was first described by Mascaro, Lecha and Torras (1979). This finding has been confirmed recently by several authors (Saylan & Saltik, 1982; Bowers & Powell, 1983; Kurkcuoglu, Atakan & Eksioglu, 1985). These authors reported the good results of thalidomide on oral and genital ulceration in Behçet's syndrome. Healing followed almost immediately after starting treatment, whereas several other treatment modalities, including steroids and cytotoxic agents, failed. Recurrence of oral and genital ulceration was not as severe or as frequent as before, when treatment was discontinued. In these reports, thalidomide was observed to be less effective in treating ocular complications and arthritis, although the drug permitted a reduction in steroid dosage in the treatment of ocular attacks.

Involvement of the nervous system is seen in about 10% of Behçet's patients and is a serious, often fatal, complication.

The effect of corticosteroids alone on the neurological complication varies, but is generally poor. Better results have been obtained when corticosteroids are combined with immunosuppressive agents, particularly chlorambucil (Inaba & Aoyama, 1982). The recently reported value of levamisole (Lavery & Pinkerton, 1985; de Merieux, Spitler & Paulus, 1981) and acyclovir (Prieto, Suarez & Civeira, 1984; Resegotti & Pistone, 1984) in Behçet's syndrome needs further evaluation.

The aetiology and pathogenesis of Behçet's syndrome are virtually unknown. However, several reports suggest that immune complexes may contribute to the systemic inflammatory process (Lehner, Almeida & Levinsky, 1978; Gupta *et al.*, 1978). Recently, Valesini *et al.* (1985) found an imbalance of T-cell subsets present in patients with Behçet's syndrome.

Studies have shown that thalidomide has an anti-inflammatory action in leprosy (Hasting, Morales & Shannon, 1976). It antagonizes the chemical mediators of inflammation and inhibits the chemotaxis of neutrophils. In addition, prolonged skin homograft survival (Hellmann, Duke & Tucker, 1965), inhibition of lymphocyte transformation (Roath, Elves & Israëls, 1963) and decreased proliferation of immunoblasts *in vivo* have been reported (Turk, Hellmann & Duke, 1966). Initially, the immunosuppressive action of thalidomide has proved of value in the treatment of the lepra reaction (Sheskin, 1965). Similar good results have been reported in chronic discoid lupus erythematosus (Barba-Rubio & Franco-Gonzàlez, 1975; Knop *et al.*, 1983), sub-acute cutaneous lupus erythematosus (Naafs *et al.*, 1982), actinic prurigo (Londono, 1973; Lovell *et al.*, 1983), Weber-Christian disease (Eravelly & Waters, 1977), and more recently, rheumatoid arthritis (Gutierrez-Rodrigues, 1984). Our patient is currently asymptomatic and leading a normal life. Our observations are restricted to one patient, and we must allow for spontaneous remission, although the prompt remission of the disease after starting thalidomide therapy does not support this view.

Thalidomide was withdrawn from the market because of its teratogenic effect. A reappraisal and further evaluation of this drug, especially in life-threatening diseases, would appear to be indicated.

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# Cholinergic dermographism

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## SUMMARY

We report a patient with cholinergic urticaria in whom stroking the skin produced a band of erythema studded with the small weals characteristics of cholinergic urticaria. This response was suppressed by pre-treatment with topical scopolamine. Light and electron microscopy of the weal showed mast cell degranulation and a moderate mononuclear cell infiltrate.

Cholinergic urticaria is common, occurring in 20% of young adults (Calnan, 1964) and accounts for 5–7% of patients presenting with urticarial eruptions (Moore-Robinson & Warin, 1968). The typical reaction is of 2–3 mm diameter weals on an erythematous flare developing in response to exercise, emotional stress or rise in body temperature (Herxheimer, 1956). Dermographism is also common, occurring in approximately 5% of otherwise normal individuals (Lewis, 1924; Kirby *et al.*, 1971) and in 8·5–13% of patients with chronic urticaria (Champion *et al.*, 1969; Moore-Robinson & Warin, 1968). It is characterized by linear wealing associated with itching in response to stroking of the skin. The development of typical cholinergic weals in response to skin stroking has been recognized previously (Baughman & Jillson, 1963; Baughman, 1964; Warin & Champion, 1974) but is uncommon and, to our knowledge, has not been investigated further. Using a spring-loaded dermographometer, linear wealing can be produced in 92% of patients with symptomatic dermographism, but not in control subjects, at a low dermographometer pressure of $3{\cdot}5 \times 10^5$ pascals (Breathnach *et al.*, 1983). In our patient, application of the dermographometer at this low pressure resulted in small cholinergic urticarial weals in a linear distribution, together with itching.

## CASE REPORT

A 29-year-old female presented in April 1984 with a 6-week history of pruritus. During stress, stroking or rubbing the skin produced intense pruritus with small monomorphic urticarial weals and a surrounding erythematous flare along the line of the stroke marks. This response occurred

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independently of generalized cholinergic urticaria and individual lesions lasted approximately 45 min. Exercise, emotional stimuli and hot baths provoked a typical cholinergic urticarial reaction, predominantly over the upper trunk, but cold, sunlight and vibration had no effect. She had no signs or symptoms of atopy and there was no family history of urticaria or atopy. The patient responded well to treatment with the combination of an H1 antihistamine, terfenadine (Triludan) 60 mg twice a day and the anticholinergic drug poldine methyl sulphate (Nacton) 4 mg three times per day. The dose of these was reduced gradually and stopped. The patient then had an episode of remission lasting several weeks, when the stress had been removed, but the lesions recurred. She currently (October 1985) has occasional episodes of mild pruritus and urticaria which is controlled by terfenadine 60 mg taken as necessary.

## INVESTIGATIONS

*Clinical tests for physical urticaria.* The patient was tested for solar urticaria with a monochromator at doses of 5–500 mJ/cm$^2$ at 300 and 307·5 nm and for cold urticaria with an ice cube applied for 15 min. Pressure urticaria was tested by the constant application of the spring-loaded calibrated dermographometer (James & Warin, 1969) for 1, 2 and 5 min at pressure increments from $0{\cdot}8 \times 10^5$ to $9{\cdot}4 \times 10^5$ pascals. (Estes & Young, 1981). The exercise test consisted of running on the spot in warm clothing till sweating was induced. For the hot bath test the patient was immersed in a bath at 42°C for 10 min. Dermographism was elicited by stroking the skin with a calibrated spring-loaded dermographometer.

*Mechanism of weal production.* To examine the role of acetyl choline in weal production, tests with a specific competitive antagonist of acetyl choline were performed. A 10 × 10 cm gauze swab was soaked in 9% aqueous hyoscine hydrochloride (scopolamine hydrochloride) and applied to the upper back for 5 min. A similar swab was soaked in distilled water and applied to a symmetrical site on the upper back to act as a control. The effectiveness of the cholinergic blockade was demonstrated by exercising the patient. Abolition of sweating on the scopolamine-treated site, but not the control, was then demonstrated using starch iodide paper. By means of the calibrated dermographometer, pressures of $0{\cdot}8 \times 10^5$, $3{\cdot}5 \times 10^5$, $6{\cdot}7 \times 10^5$ and $9{\cdot}4 \times 10^5$ pascals were each applied as a 6 cm vertical stroke to both scopolamine pre-treated and control sites on the upper back. This procedure was repeated on three occasions. Counts of the resulting small weals were carried out by two independent observers 10 min after the challenge.

*Histology and ultrastructure.* Using a 3 mm punch under local anaesthesia (2% lignocaine without adrenalin), biopsies were taken for mast cell counts and histopathology in normal forearm skin and a cholinergic weal on an adjacent site. The weal was induced by means of the calibrated dermographometer set at $6{\cdot}7 \times 10^5$ pascals, and biopsied 2 min after its appearance. No previous dermographometry testing had been performed on this forearm.

Both samples were bisected and immersed immediately in half-strength Karnovsky fixative containing 0·1 M cacodylate buffer, 5% sucrose and 0·05% calcium chloride. After 4 h of primary fixation at room temperature, the samples were washed several times in cacodylate buffer and then subjected to secondary fixation in 1·3% osmium tetroxide in 0·067 M collidine buffer, on ice for 2 h. The tissues were then dehydrated in ethanol and embedded in Epon. Semi-thin (1 μm) sections were stained with basic fuchsin and methylene blue according to the method of Huber, Parker and Odland (1968) modified by Eady (1985).

The total number of mast cells, identified as spindle-shaped or round mononuclear cells containing bright red or purple intracytoplasmic granules, was counted by light microscopy in sections from two blocks from each biopsy sample, using the technique of Eady *et al.* (1979). The number of mast cells undergoing degranulation was also counted. Mast cell degranulation was deemed to have occurred if free granules could be seen in the extracellular space immediately surrounding the mast cells. In addition, the sections were examined for features of acute inflammation, including vessel dilatation, oedema, fibrin deposition and the presence of inflammatory cells.

When the patient was in remission, 0·05 ml of histamine (10 μg/ml) and 0·1 ml of acetyl choline (20 μg/ml), both in buffered normal saline as a diluent, were injected intradermally. The resultant weals were biopsied at 2 min, the tissue fixed in 10% formal saline, paraffin embedded, and 6 μm sections stained with haematoxylin and eosin.

## RESULTS

The following routine investigations were normal: haemoglobin, white cell count, ESR, blood film, urea and electrolytes, liver function tests, blood sugar, cold agglutinins, Rose Waaler test, VDRL, TPHA, ANA, C3, C4, C1 esterase inhibitor and chest X-ray. Tests for solar urticaria, cold and pressure urticaria were negative. The exercise test resulted in erythema and few weals 3–4 mm in diameter over the trunk; the hot bath test was negative.

Any stressful situation promoted by emotion, such as a medical interview, resulted in a typical cholinergic urticarial eruption with small weals and an erythematous flare over the neck and upper chest. The response to stroking of the skin produced by the dermographometer was of irregular linear wealing giving a beaded appearance with a surrounding erythematous flare at pressures of $0{\cdot}8 \times 10^5$, $3{\cdot}5 \times 10^5$, $6{\cdot}7 \times 10^5$ and $9{\cdot}4 \times 10^5$ pascals (Fig. 1). The patient had symptoms of intense itching at the site of the dermographic response. The wealing response was

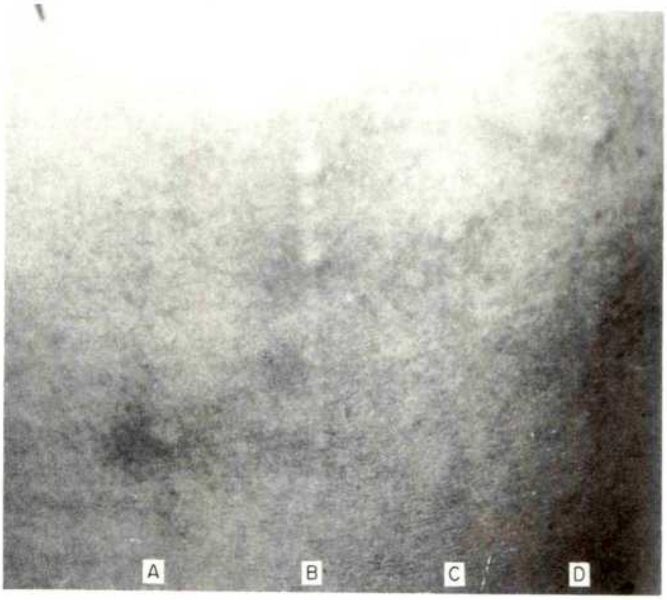

FIGURE 1. Photograph showing weals surrounded by a flare, in a linear distribution at sites of dermographometer strokes at pressures of (A) $0{\cdot}8 \times 10^5$; (B) $3{\cdot}5 \times 10^5$; (C) $6{\cdot}7 \times 10^5$, and (D) $9{\cdot}4 \times 10^5$ pascals.

TABLE 1. Effect of scopolamine on weal count

| | No. of weals/100 cm² | |
|---|---|---|
| Test | Scopolamine | Water |
| 1 | 4 | 8 |
| 2 | 3 | 15 |
| 3 | 4 | 18 |

TABLE 2. Numbers of mast cells and degranulated cells in biopsies from normal forearm skin (pre-challenge) and a cholinergic weal (post-challenge)

| Sample | Total no. mast cells/section | Mast cells/mm² | Total no. degranulated cells/section | Percentage of cells degranulating |
|---|---|---|---|---|
| Pre-challenge | | | | |
| Block 1 | 106 | 65·2 | 16 | 13·0 |
| Block 2 | 72 | 41·4 | 14 | 16·3 |
| Mean | | 53·5 | | 14·7 |
| Post-challenge | | | | |
| Block 1 | 109 | 64·5 | 50 | 31·4 |
| Block 2 | 117 | 55·5 | 63 | 31·2 |
| Mean | | 60·0 | | 31·3 |
| Normal range (Coutts *et al*, 1981) | | 34·8–66·4 | | 10·1–13·5 |

reduced significantly by the prior topical application of scopolamine hydrochloride. Table 1 shows the results of weal counts on the scopolamine pre-treated and water pre-treated sites on each of the three occasions. The one tailed paired *t*-test showed that there was a significant difference between the weal counts in the two sites ($P < 0{\cdot}05$).

*Mast cell counts.* The total number of cells and percentages undergoing degranulation are shown in Table 2. The population density of mast cells in pre-challenge skin and the weal were within the normal range. However, increased mast cell degranulation occurred in the post-challenge sample.

*Histology.* During the active phase, the pre-challenge skin sample contained moderate numbers of mononuclear cells (macrophages, lymphocytes and fibroblasts), surrounding blood vessels in the sub-papillary plexus. In the post-challenge sample, oedema was present within the dermis and epidermis. Numerous keratinocytes were vacuolated (Fig. 2), a characteristic epidermal change in weals (Eady *et al.*, 1981). In addition, there was vascular dilatation,

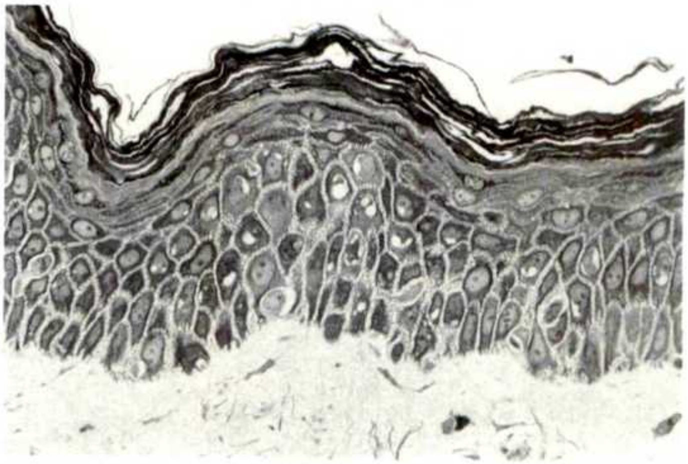

FIGURE 2. Photomicrograph of epidermis in a dermographic weal. Note intracellular vacuolation and apparent expansion of the intercellular spaces. (Basic fuchsin and methylene blue; original × 330.)

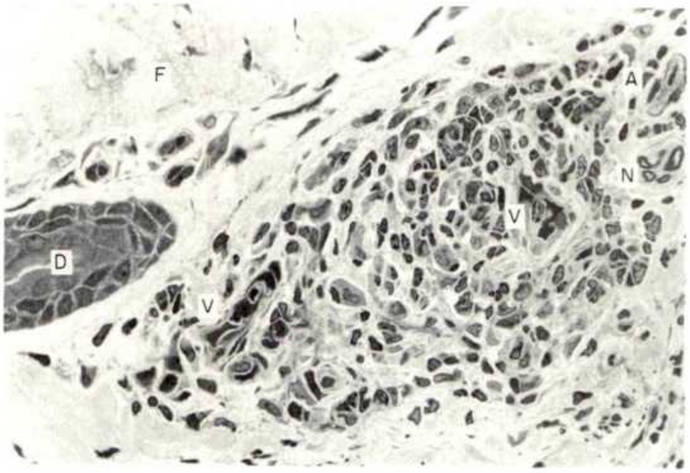

FIGURE 3. Photomicrograph of dermal cellular infiltrate in a dermographic weal. Note cluster of mononuclear cells (chiefly macrophages and lymphocytes around dilated venules (V) and close to a sweat duct (D), ascending arterioles (A) and myelinated nerve (N). Fibrin deposition (F) is evident in the connective tissue at the periphery of the cellular infiltrate. (Basic fuchsin and methylene blue; original × 330.)

affecting arterioles, venules and lymphatics, and fibrin deposition. There was an increase in the numbers of peri-vascular lymphocytes and macrophages in both papillary and reticular dermis, but noticeably adjacent to appendages (Fig. 3). No eosinophils, neutrophils or basophils were observed in any of the sections.

During the remission phase the haematoxylin and eosin tissue section of the histamine-induced weal showed slight endothelial swelling only, and that of the acetyl choline-induced weal endothelial swelling with only occasional perivascular mononuclear cells. Chloroacetate esterase stains of both of these induced weals demonstrated small numbers of perivascular mast cells in the dermis. There was no evidence of mast cell degranulation.

## DISCUSSION

We have demonstrated a little known variant of cholinergic urticaria and dermographism and shown that, as in classical cholinergic urticaria, it is mediated partly by acetyl choline. The dermographic response was obtained at all dermographometer pressures, including the lowest, and consisted of weals identical to those of cholinergic urticaria evoked in the patient by emotion and exercising. The weals developed within minutes of stroking the skin both with the dermographometer and as a response to scratching and lasted for approximately three quarters of an hour.

The contribution to this response by acetyl choline is demonstrated by the significant reduction in weal counts between the area pre-treated with the topical anticholinergic and the control. Acetyl choline is a known mast cell degranulator and histamine releaser (Cho & Ogle, 1979; Blandina *et al.*, 1980), although we could not detect mast cell degranulation in the weal produced by intradermal injection of acetyl choline using the chloracetate esterase stain.

The histological findings were surprising, the pre-challenge skin showing a moderate perivascular mononuclear cell infiltrate and histology of the weal demonstrating a dense dermal infiltrate, again consisting chiefly of mononuclear cells. Winkelmann (1986a) has demonstrated a marked perivascular lymphocytosis in normal unstimulated skin of a patient with dermographism, although James *et al.* (1980) stated that histology of normal skin in physical urticaria of various types is unremarkable.

More information on the histology of unstimulated skin in patients with dermographism is needed. During remission there was only a minimal infiltrate in the histamine and acetyl choline weals in our patient, suggesting that the histology of her skin that was not injected was normal. It is possible that a mononuclear cell infiltrate is a pre-requisite for the production of dermographism. The dense perivascular lymphocytic dermal infiltrate in the cholinergic dermographism-induced weal is difficult to explain. A similar infiltrate has been demonstrated in early spontaneous weals in dermographic patients (Winkelmann, 1986a) and in cholinergic weals. However, the more usual histological picture in both cholinergic urticaria and dermographism is of an early polymorphonuclear cell infiltrate with a perivascular lymphocytic infiltrate in later lesions (Winkelmann, 1986a, b).

These histological observations have been carried out so far on small numbers of patients only, and further studies are needed to establish the significance of the different infiltrates and their time sequence.

## ACKNOWLEDGMENTS

We are grateful for the assistance of the Herbert E.Dunhill Trust. Andrew Kennedy provided excellent technical assistance.

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# Occupational high-pressure injection injury

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## SUMMARY

A case of occupational high-pressure injection injury and details of its successful management is recorded, and the literature on this type of injury reviewed.

Although high-pressure injection injuries have been recognized for over 50 years as an occupational hazard, they are rarely reported in spite of major morbidity and rare rehabilitation (Vinogrodov, 1936). This report would appear to be the first report of such an injury as a farming hazard, and is unusual in the site affected.

## CASE REPORT

In March 1984, a healthy 33-year-old local farmer was referred because of a presumed cellulitis of penis and scrotum. He had sustained an injury at work when a hydraulic lubricating hose under high pressure came loose, but had concealed the site and type of injury for 10 days prior to hospital admission. A stream of oil had hit his body below the pubis, penetrating his clothes. A small pit on the under side of the penis was the point of entry. The penis itself was grossly enlarged and distorted. The skin was turgid, yellowish in colour and cool to touch. Sensation was apparently normal, but the patient experienced constant, painful erections, frequently disturbing sleep. Swelling continued to spread over the pubis and natal cleft, causing acute discomfort.

Incisional biopsies down to Buck's fascia were taken and showed numerous vacuoles and minute cysts, associated with a moderate degree of inflammation, granulation tissue and fibrosis. Infiltrates consisted of lymphocytes, plasma cells and histiocytes; multinucleated foreign body giant cells were rare and eosinophils were absent. An oil red O stain showed fat droplets in the vacuoles and cystic spaces, and polarized light showed refractile crystals in the fat droplets. Immunofluorescence studies showed granular deposits of C3 at the dermo-epidermal junction throughout, as seen in a variety of immunological diseases, which were also present in dermal arterioles along with small quantities of IgG and IgM.

On the basis of these findings, a diagnosis of lipid granulomatosis was made. High-dose oral steroid therapy was commenced, and within a week the swelling was much reduced and painful

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erections had ceased. However, reduction of the steroid dose resulted in recurrence of the patient's problems, including a continuing subcutaneous spread of the offending material. Because of this, plastic surgery was performed (Agha, 1978). At operation, thick, yellow indurated tissue was filleted down to Buck's fascia. This damaged tissue covered the testicles, the entire shaft of penis and suspensory ligament of the penis up to the suprapubis and to the perineum caudally. After 750 mg fluorescein had been injected and replacement flaps examined by Wood's light, further excision of tissue down to the suspensory ligaments was deemed necessary. New scrotal pockets were effected and closure done with 4-0 DEXON and haemovac drains. Recovery was slow, but complete, and normal sexual function resumed by 4 months post-operation.

## DISCUSSION

In 1937, Rees described the inadvertent injection of fuel oil under pressure into subcutaneous tissues. Since that time less than 60 cases of high-pressure injection injury have been reported in print. Although the majority of reported cases have been industrial accidents (Scott, 1983), the author's practice is largely rural and the farmers, open to this class of injury, being self-employed, cannot claim for industrial injury. Their problems have gone thus unnoticed and unreported.

The injury site is usually pin-hole size as lubricating nipples have an average diameter of 1–2 mm. The injury occurs when the nipple is inserted incorrectly, pressure turned on, and the lubricant sprayed out at pressures in excess of 2000 p.s.i. The force can be over 3000 p.s.i. with the engine revving, and up to 1000 p.s.i. when idling (Herrick, 1980). The injury is almost as severe through gloves or clothing as on bare skin. The speed of injection can be up to 1000 feet/s at distances of 1–2 inches from the point of breaking the skin. The pressures can be translated into average 5–10 000 kilograms at 3–5 cms.

The patient is aware of the injury immediately, but there is a latent anaesthetic period, followed by gradual onset of swelling, pain and ischaemic problems (Gerberman, 1975). To date, all reported cases have involved fingers or palms, except one case where an activated air-pressure nipple was put next to the tongue with subsequent swelling and temporary loss of function. The tools or machinery involved vary extensively. They include paint spray guns, hydraulic pumps, diesel-fuel injectors, compressed air lines, presses for injection moulding of plastics, and, in the case of farmers, the hydraulic injection systems incorporated into their tractors. Farmers use these systems to lubricate their non-power-driven machinery. This work is often done in the open field, at speed, and alone. In spring and autumn, agricultural workers may be averaging an 18 h day, and the hazards are obvious. The mechanism of injury is not established, but is probably vessel spasm from pressure at the point of entry, and subsequent venous obstruction from the progressive tissue swelling, the severity of the injury being directly proportional to the magnitude of the pressure, distance from the source, the materials injected and the time until initial decompression.

The quality of subsequent care and the appreciation of the pathophysiological problems are obviously paramount for return of function, which has to date been poor.

The most irritant material is Turpentine, a true tissue fixative, and the least irritant is 'chock oil' (95% potable water with 5% miscible oil), with lubricating grease giving little local reaction. However, all continue to spread in the subcutaneous tissue as shown in the case described.

Many greases in commercial use contain both radiopaque lead thickening agents, and calcium complex agents to prevent breakdown of the lubricating film under high-compression loads.

One half of the internationally listed greases contain lead for this purpose. Pre-operative X-rays are thus extremely valuable if grease is the offending substance. If the grease is not radiopaque then xero-radiology will delineate the affected tissues (Crabb, 1981).

As many of the injuries are produced by hand-held machines, the site of injury is usually the non-dominant index finger. The treatment, as suggested by Kaufman (1968), is 5 min elevation of the affected part, followed by tourniquet.

The offending material in this present case was Hydraulic 50, a lubricating oil which is a mixture of paraffinic and naphthenic hydrocarbons with trace amounts of aromatic hydrocarbons and additives (antirust, antifoaming and antiknock chemicals) all in small amounts (product data, Imperial Oil Ltd). The accompanying brochure included skin and eye contact procedures, describing slight irritation but no tissue damage to conjunctiva.

Historically, oil granulomata have been known for many years. In 1937, Rees described a case caused by the fuel-injection mechanism of a diesel engine, and in 1939, Smith reported a case caused by a high-pressure grease gun. In 1963, Gruner reported cases caused by the high-pressure spray gun, at that time a new industrial tool. The histology of oil granulomata was described by Hess (1925), Dickson (1976) and Vinogradov (1936), as self-inflicted wounds by Russian males to avoid conscription.

The principles of treatment for this type of injury have been outlined by Herrick (1980). These can now be amended slightly and include rapid referral to a plastic surgeon, X-ray or, if necessary, xero-radiology, obtaining a chemical breakdown of the offending substance, and decompression and complete cleansing of the affected area, possibly under general anaesthesia. If a limb is affected, this is raised for 5 min, and a tourniquet is applied.

Tetanus toxoid and human tetanus immune globulin may be needed, and high-dose oral steroids may be valuable, particularly if there has been delay in treatment. Careful observation is needed as further decompression, and excision of all affected tissue, may have to be repeated. In the case of digits, amputation may be needed, but the thumb must be preserved if at all possible.

## ACKNOWLEDGMENTS

Dr J.J.San Vicente, urologist, carried out the biopsies, Dr John Klasen read the histology, and Dr Robert Carter, plastic surgeon, carried out necessary surgery.

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### Poikiloderma vasculare atrophicans granulomatosa

MADAM, A 58-year-old woman was seen at our department with a 6 year history of persisting skin lesions on the breasts, natal cleft and right forearm. At these sites large polymorphous plaques with reticular hyperpigmentation, atrophy and telangiectases were present (Fig. 1). The lesion on the right forearm had a more yellowish brown hue (Fig. 2). Otherwise, the physical examination was normal. The clinical picture was compatible with the diagnosis of poikiloderma vasculare atrophicans. However, the discoloration in one of the lesions was considered to be unusual for this entity.

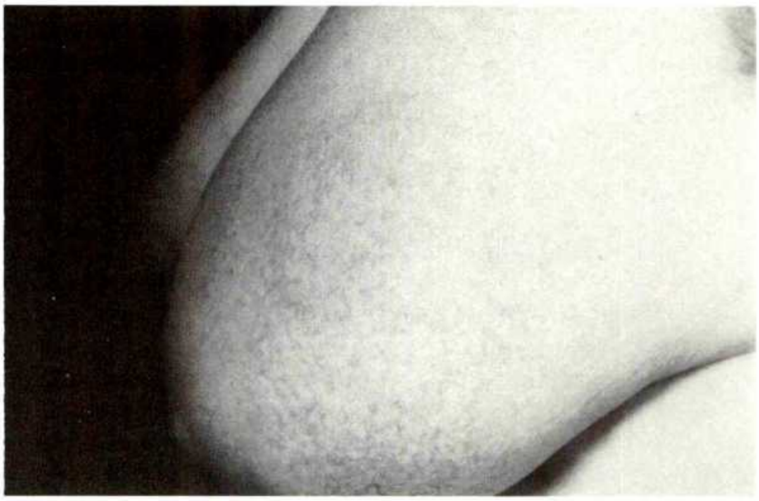

FIGURE 1. Poikiloderma vasculare atrophicans demonstrating reticular hyperpigmentation, hypopigmentation, atrophy and telangiectases.

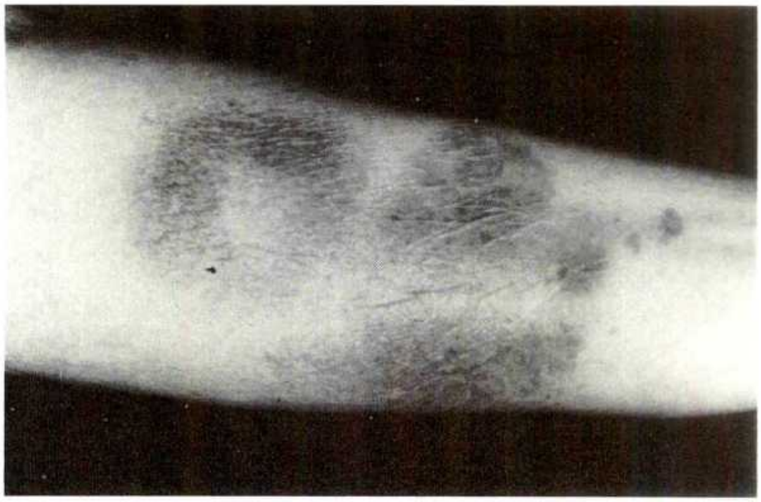

FIGURE 2. Poikiloderma on the forearm. The lesion had a more yellowish brownish hue.

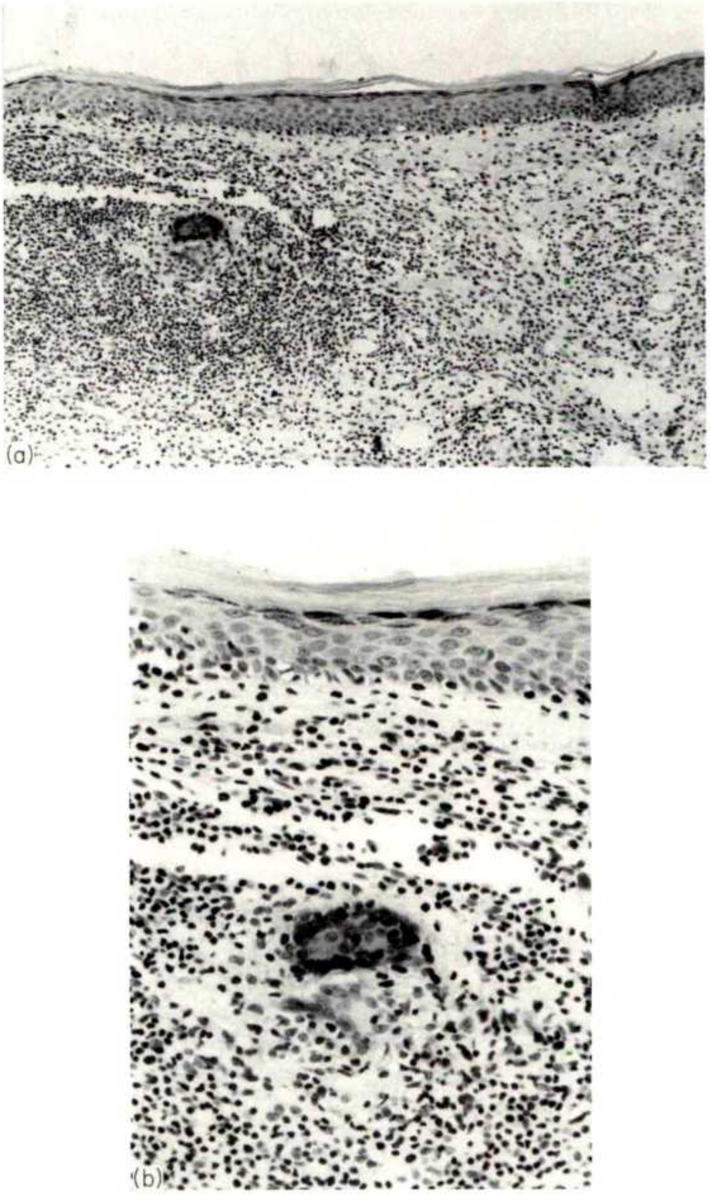

FIGURE 3. Lesion on the forearm, showing an atrophic epidermis with hydropic degeneration in the basal layer. The infiltrate in the dermis consists of lymphocytes, histiocytes, eosinophils, atypical mononuclear cells and giant cells. (a) Original × 100; (b) original × 250.

Histological examination of the lesion on the right forearm was carried out (Fig. 3a and b). The epidermis was atrophic and the basal layer showed extensive hydropic degeneration. In the dermis a dense infiltrate was seen, composed of lymphocytes, some eosinophilic granulocytes, histiocytes, atypical mononuclear cells, focal solitary giant cells or non-caseating granulomas. In some places, lymphocytes had penetrated the epidermis. No foreign body material was detected with the polarizing microscope. Some granulomas were seen in relation to destroyed hair follicles or their contents; most of them, however, had no such relationship, even in serial sections.

The patient refused therapy. However, 2 years later she returned. Apart from some enlargement of the lesion on the forearm, the clinical picture was similar to that of 2 years earlier. A new biopsy at this time revealed a histological picture identical to the first. Indirect immunoperoxidase staining of cryostat sections with monoclonal antibodies against T and B cell markers and direct immunofluorescence with antibodies against immunoglobulins, showed that the subepidermal lymphocytic infiltrate was 90% of T cell origin, and that in this T cell population both T helper and suppressor cells were present. The ratio of T helper/T suppressor cells was consistent with a reactive infiltration and varied in the two subsequent biopsies from 0·6 to 2·0.

Full blood and urine investigations, intermediate strength PPD and X-ray examination of the chest did not reveal any abnormality. PUVA therapy was started. Three months after starting therapy, the lesions have improved markedly.

Poikiloderma vasculare atrophicans may occur in association with several skin disorders. In this patient the onset of the skin disease in the fifth decade, the typical large plaques situated on the trunk and the dense lymphocytic infiltrate are all indicative of the Lane type of poikiloderma vasculare atrophicans, a manifestation of large plaque para-psoriasis (Altman, 1984). Progression to cutaneous lymphoma has been reported in between 10% and 20% of the patients with this disorder (Lambert & Everett, 1981). A notable finding is the presence of epitheloid granulomas in association with otherwise characteristic lesions of poikiloderma vasculare atrophicans.

Giant cell infiltrates are exceptionally rare in mycosis fungoides. However, they have been reported (Ackerman & Flaxman, 1970; Vignon-Pennamen *et al.*, 1983). Giant cells originate from activated monocytes. Monocytes and macrophages are present in the cutaneous infiltrates of early mycosis fungoides. In the more advanced stage and in extracutaneous disease they are much rarer (Norris, 1981).

At present, we can only speculate regarding the significance of giant cells in the pathogenesis of poikiloderma vasculare atrophicans and mycosis fungoides.

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## Acne and unemployment

MADAM, The physical and psychological problems associated with chronic skin disease are well known. In the past 2 years we have had the opportunity of investigating unemployment in patients with clinical acne. We investigated unemployment levels among 625 acne patients (327 males, 268 females) aged between 18 and 30 years, seen over a 9-month period. Unemployment among a control group of 625 subjects with a virtually identical age range and sex ratio, selected at random from general practitioner records for the same catchment area, was used for comparison. We did not examine the control subjects. Neither group included students (full or part-time), patients from overseas or housewives.

Unemployment levels were significantly greater among acne patients of both sexes than in the controls; 16·2% compared with 9·2% in males—76% higher, and 14·3% compared with 8·7% in females—64% higher ($P < 0{\cdot}001$; chi squared test). It was not possible to analyse factors such as social status, academic background or intelligence, since data were not readily available.

These figures highlight a further psychological feature of acne and should stress to the physician the need to treat acne promptly and adequately. There is no reason why, in 1986, all acne patients should not respond well to available therapy.

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## Lasers and psoriasis

MADAM, Our observations concerning the use of an argon laser on psoriatic plaques in 19 patients (Harrison, Walker & Davies, 1985) have supported those of Békássy and Åstedt (*British Journal of Dermatology*, 1986, **114**, 489), who used a carbon dioxide laser. Traumatic methods including cryotherapy, laser therapy and surgery can certainly improve psoriasis. However, the tolerance of the patient, complexity of equipment used, or inability of the method to deal with anything other than very small areas of chronic, stable plaque-type psoriasis, at present limit these therapies to interesting research techniques. Our results with traumatic methods of treatment for psoriasis have led us away from laser therapy, even though it can clear small areas of psoriasis, and we occasionally use surgical methods of treatment, (Kiil, Kiil & Søgaard, 1985). Shave excision can be employed for the occasional patient with localized areas of stable plaque-type psoriasis, dermabrasion may remove psoriasis from sites such as elbows and knees, and we are currently assessing the results of dermabrasion on palmoplantar pustular psoriasis.

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# Book Reviews

**Cutaneous Photobiology.** W.FRAIN-BELL (1986) Oxford: Oxford University Press. Pp. 244. Price £45.00.

Dr Frain-Bell has produced an excellent review of the clinical aspects of photobiology. He has long been acknowledged as an expert on the subject, having for many years directed the dermatological photobiology unit at Ninewells Hospital in Dundee. The whole field of photobiology is covered in his clear review, which includes a brief discussion of the effects of light on normal skin in the introduction, followed by a description in detail of the features and treatment of all the photodermatoses, a brief section on the eyes and a comprehensive review of the use of light itself in the therapy of cutaneous disease. The book is completed by a section on investigation of light sensitivity. The chapters are supported by many excellent clinical pictures and relevant references throughout and there is an excellent index.

The advantages of the book are its comprehensive coverage of the whole subject of clinical photobiology at a level which is useful and convenient to the general dermatologist and informative to the photobiologist, while at the same time being a compact manageable volume. It is a valuable addition to the range of texts on photobiology now available, being much more of a clinical handbook than the others. Its major disadvantages are a slightly circumlocutory style of writing and an occasional failure to make points definitively, but these detract little from the fact that all the necessary information has been collated and offered in a clear, logical layout which must adorn the bookshelves or briefcases of all who are interested in photobiology.

J.L.M.Hawk

**Urticaria.** BEATE M.CZARNETZKI (1986) Berlin: Springer. Pp. 189. Price DM 168.

This is the best book on urticaria that I have come across recently. Its uniformity of style puts it ahead of any volumes made up of papers given at conferences on urticaria: it is well written, well presented, and well bound. The tables are good, the diagrams are good, and the colour pictures are good. Perhaps it is possible to parody its 'thorough' style, but there can be no doubts about its solid virtues and its success in fulfilling its stated aim of being an 'in-depth review of all major basic and clinical aspects of urticaria'. I see it as a worthy successor to the much respected monograph on urticaria written more than a decade ago by Warin and Champion.

The book has a few lighter moments too. For some reason, I found the picture on page 68 funny. It shows a man lying on his stomach with an apparatus perched on his back transmitting pressure through rods onto the skin. Its purpose is to investigate patients with pressure urticaria but to my eye it looked oddly mystical, rather like a bed of nails lying on a person.

There are also some minor irritations. Why are the references for all of the chapters grouped together at the end of the book? My practice is to look up some references as I go and this arrangement makes for a lot of page turning. Similarly, descriptions of the treatment of the various types of urticaria are confined to a single chapter. I found it tiresome, for example, to read about cholinergic urticaria on page 75, and then to have to move to page 145 to find out how to treat it. I also wondered how many British dermatologists would agree that prick tests are an 'extremely valuable diagnostic tool' providing important information about the cause of urticaria.

But these are minor quibbles: this excellent book more than earns its place in any departmental library.

J.A.SAVIN.

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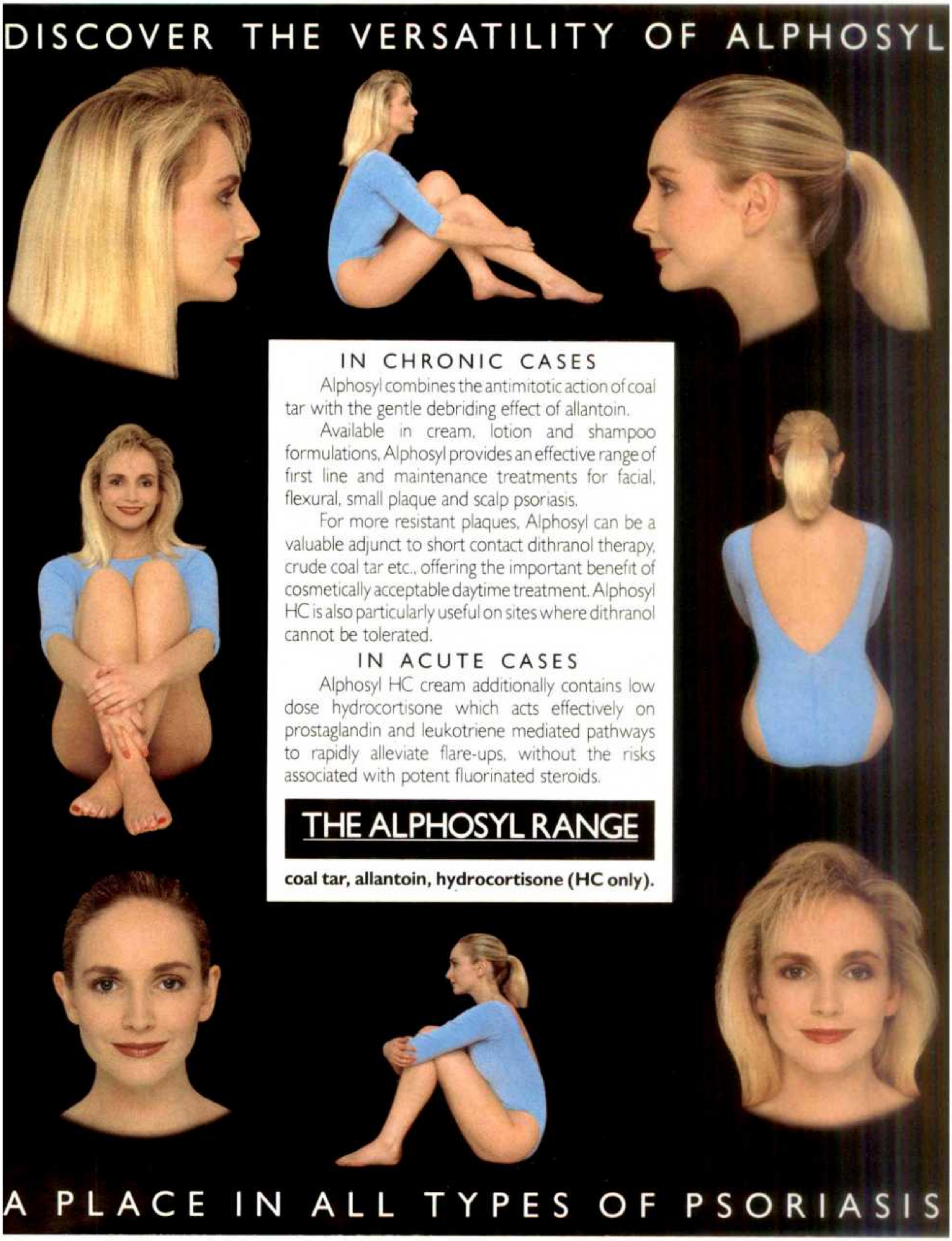
# *News and Notices*

### Department of Dermatology, University of Glasgow—Dermatopathology Course

The Annual Dermatopathology Course held by the Department of Dermatology in Glasgow will take place from Monday, 27 March to Friday, 3 April 1987. This course covers all major aspects of dermatopathology and is suitable for both pathologists and dermatologists. The course is restricted to 24 members to allow for individual tuition.

Early application is advised and individuals wishing to attend the course should contact Mrs W.E. Scott, Department of Postgraduate Medical Education, Glasgow University, Glasgow G12 8QQ.


DISCOVER THE VERSATILITY OF ALPHOSYL
A PLACE IN ALL TYPES OF PSORIASIS

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## IN ACUTE CASES

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**coal tar, allantoin, hydrocortisone (HC only).**

**cribing Information** ALPHOSYL CREAM, LOTION & APPLICATION PC. **Uses** Treatment of psoriasis. Application PC. Psoriasis of the scalp. **Dosage & Administration** Apply liberally 2 to 4 times da
lication PC. Apply shampoo to wet hair, massage to lather and rinse. Repeat if necessary. **Contra-indications, warnings etc.** Sensitivity to coal tar. Discontinue if irritation occurs. External use only. **Presentatic**
ıed alcoholic extract of coal tar 5%, allantoin 2% (Application PC 0.2%). **Basic NHS Cost** Cream 75g, £1.56. Lotion 250ml, £2.27. Application PC 60g, £0.48. **Product Licence Nos.** Cream 0036/50C
on 0036/5008. Application PC 0036/5005. ALPHOSYL HC. **Uses** Treatment of inflamed psoriasis. **Dosage & Administration** Apply 2 to 4 times daily. **Contra-indications, warnings etc.** Should not be used in sk
tions. Should not be used extensively in pregnancy. Avoid long-term use in infants. **Presentation** Contains refined alcoholic extract of coal tar 5%, allantoin 2%, hydrocortisone PhEur 0.5%. **Basic NHS cost** 3C
). 45g, £2.41. **Product Licence No.** 0036/0026. Further information is available from Stafford-Miller Limited, Professional Relations Division, Hatfield, Herts. AL10 0NZ.

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minocycline hydrochloride

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**Abbreviated Prescribing Information. Dosage:** Adults and children over 12 years. One 50 mg tablet twice daily. Minocin may be taken with meals. Treatment of acne should be continued for a minimum of six weeks. **Contra-indications:** Known tetracycline hypersensitivity. Systemic lupus erythematosus. **Precautions:** Absorption is impaired by iron and antacids containing calcium, magnesium and aluminium salts. **Warnings and side effects:** Gastro-intestinal disturbances are the side effects most commonly reported. A yellow to brown discoloration of teeth is a possible sequela of treatment during pregnancy or early childhood (up to 8 years of age). **Presentation:** Beige embossed tablets containing 50 mg of minocycline as the hydrochloride, supplied in blister strips of 28 tablets. 3 strips to a carton. **Legal Category:** POM. PL 0095/0062. PA 37/7/4. **Basic NHS Price:** 48.5p per day at recommended b.d. dose (Price correct at time of printing). Full prescribing information available on request. Minocin is a trademark.

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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**Abbreviations and units.** Full names with uncommon abbreviations must be given with the first mention, new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

| | | | | | |
|---|---|---|---|---|---|
| gram(s) | g | minute(s) | min | molar | mol l |
| kilogram(s) | kg | centimetre(s) | cm | millilitre(s) | ml |
| milligram(s) ($10^{-3}$g) | mg | second(s) | s | gravitational acceleration | *g* |
| microgram(s) ($10^{-6}$g) | μg | cubic millimetre(s) | $mm^3$ | micrometre(s) | μm |
| nanogram(s) ($10^{-9}$g) | ng | millimetre(s) | mm | per cent | % |
| picogram(s) ($10^{-12}$g) | pg | millicurie(s) | mCi | isotopic mass number placed as | $^{131}$I |
| hours(s) | h | milliequivalent | mmol | | |

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There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

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# British Journal of Dermatology, Volume 115, Number 3, September 1986

CONTENTS

PAGE

**Invited Editorial**

Hidradenitis suppurativa: an androgen-dependent disorder 259
F.J.G.EBLING

**Laboratory and Clinical Investigations**

A double-blind controlled cross-over trial of cyproterone acetate in females with hidradenitis suppurativa 263
P.S.MORTIMER, R.P.R.DAWBER, MARY A.GALES AND R.A.MOORE

Control of hidradenitis suppurativa in women using combined antiandrogen (cyproterone acetate) and oestrogen therapy 269
R.S.SAWERS, VALERIE A.RANDALL AND F.J.G.EBLING

Photosensitivity due to retinoids: clinical and laboratory studies 275
J.FERGUSON AND B.E.JOHNSON

Potentiation of the generation of reactive oxidants by human phagocytes during exposure to benoxaprofen and ultraviolet radiation *in vitro* 285
R.ANDERSON AND H.A.EFTYCHIS

Ionophore A23187 stimulates arachidonic acid release and cyclic GMP accumulation in pig epidermis 297
N.KATO, H.SUYA, T.AOYAGI, J.R.TAYLOR AND K.M.HALPRIN

Immunopathological and ultrastructural findings in human allergic and contact irritant dermatitis 305
C.M.WILLIS, E.YOUNG, D.R.BRANDON AND J.D.WILKINSON

Fine needle aspiration cytology in dermatology: a clinicopathological appraisal 317
D.N.SLATER AND G.REILLY

A study of HLA antigen association in localized and generalized granuloma annulare 329
R.FRIEDMAN-BIRNBAUM, O.GIDEONI, R.BERGMAN AND S.POLLACK

Animal models for percutaneous penetration: assessing contamination of the experimental system 335
C.L.GUMMER AND H.I.MAIBACH

Seborrhoeic eczema—a disease entity or a clinical variant of atopic eczema? 341
P.PODMORE, D.BURROWS, D.J.EEDY AND C.F.STANFORD

Immunogold labelling of epidermal Langerhans cells on tissue sections of normal human skin 351
G.DE PANFILIS, G.C.MANARA AND C.FERRARI

**Case Reports**

Fixed drug eruption due to paracetamol 357
R.H.MEYRICK THOMAS AND D.D.MUNRO

Periungual and subungual arteriovenous tumours 361
S.M.BURGE, R.BARAN, R.P.R.DAWBER AND J.L.VERRET

Thalidomide in the treatment of neuro-Behçet's syndrome 367
C.G.RAMSELAAR, R.M.BOONE AND H.C.KLUIN-NELEMANS

Cholinergic dermographism 371
S.C.MAYOU, A.KOBZA BLACK, R.A.J.EADY AND M.W.GREAVES

Occupational high-pressure injection injury 379
J.C.MACAULAY

**Correspondence**

Poikiloderma vasculare atrophicans granulomatosa 383
P.C.M.VAN DE KERKHOF, B.E.W.L.VAN HUYSTEE, U.J.G.M.VAN HAELST AND M.J.J.T.BOGMAN

Acne and unemployment 386
W.J.CUNLIFFE

Lasers and psoriasis 386
P.V.HARRISON

**Book Reviews** 387

**News and Notices** 388

Volume 115, Number 4, October 1986

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# British Journal of Dermatology

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# British Journal of Dermatology

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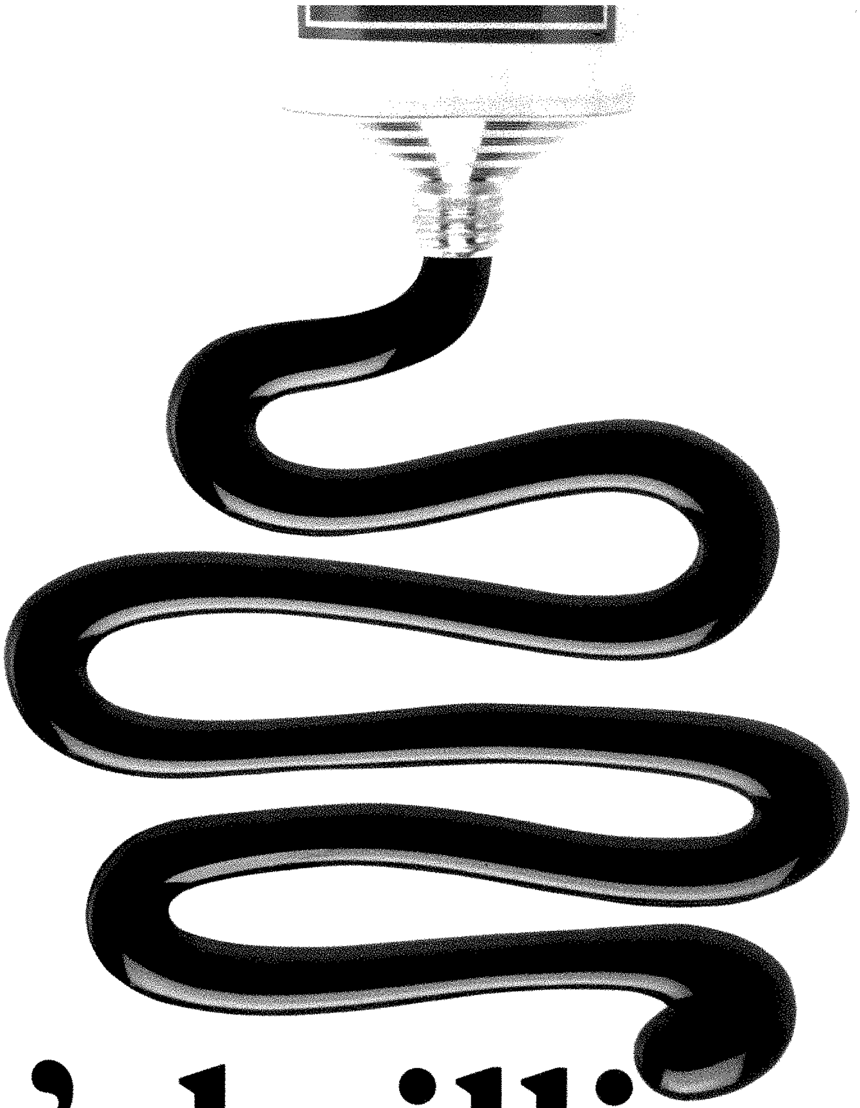

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**Prescribing Information**

**Use:** An aid in the topical treatment of venous and other types of skin ulcers.

**Directions:** Clean and dry the ulcer site, apply and smooth the Variclene gel over the affected area. Cover with a sterile dressing or pad and, where appropriate, apply compression bandaging. Repeat as required at intervals of not more than seven days.

**Contra-indications, Warnings, etc:** Avoid contact with clothing and linen. Occasionally, transient stinging on application may occur. In cases of arterial or extensive, painful venous ulceration, this may be prolonged, when treatment should be discontinued until greater tolerance has been achieved. Keep away from the eyes.

**Presentation:** 50g tube **Product Licence:** 0173/0015 (PA 278/10/1)

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Green aqueous gel containing 0.5% w/w Brilliant Green BP, 0.5% w/w Lactic Acid BP

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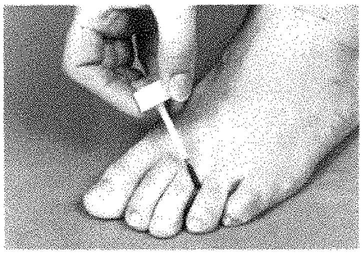
# Monphytol

**boric acid, chlorbutol,**
**methyl undecylenate, propyl undecylenate, salicyclic acid (free and methyl ester)**

## passes the acid test for athlete's foot

## After many years of success...

Monphytol is a non-messy colourless paint. When brushed on to the infected area it penetrates thoroughly to provide prompt relief and healing. Occasional application will prevent reinfection.



## ...its activity is no longer empirical

A recent study has discovered a cellular mechanism which could explain how certain acids (including the salicylic acid in Monphytol) are effective anti-fungal agents.[1]

# Monphytol®

for athlete's foot

Simple ● Effective ● Inexpensive

**MONPHYTOL**

**Presentation**: A colourless, rapidly drying, non-greasy paint containing boric acid 2%, chlorbutol 3%, methyl undecylenate 5%, propyl undecylenate 1%, salicylic acid (free and methyl ester) 31%

**Uses**: Monphytol is indicated for chronic paronychia, intertrigo erosio interdigitalis, tinea pedis, tinea circinata, tinea unguium

**Dosage and administration**: Four times a day, moisten brush with Monphytol and apply to the affected parts, reaching gently into the folds of the skin. Treatment should be repeated from time to time after the condition has subsided to prevent reinfection

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**Pharmaceutical precautions**: Store away from heat

**Legal category**: P

**Package quantities**: Bottles containing 18 ml with brush fitted in cap

**Further information**: Nil

**Product licence number**: 0118/5010

**Basic NHS price**: £0.57 per 18 ml bottle

*Reference*

1 Krebs HA, Wiggins D, Stubbs M, Sols A, Bedoya F. Studies on the mechanism of the anti-fungal action of benzoate. Biochem J. 1983; **214**: 657-63

Monphytol is a trademark

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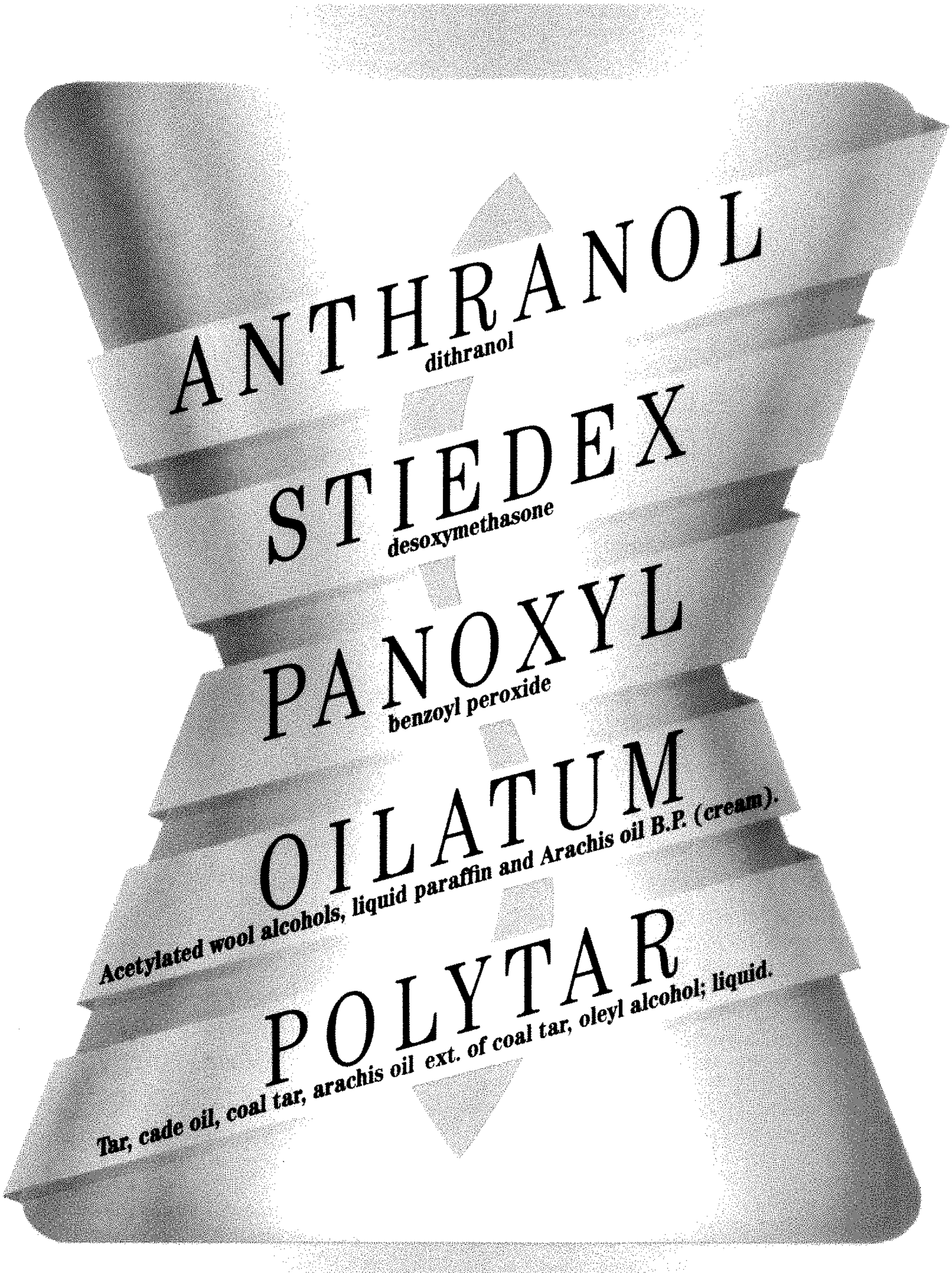
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# SYMPOSIUM ANNOUNCEMENT

## Skin Allergy Symposium

## 21st November 1986

## Café Royal, Piccadilly, London

**Chairmen:** Professor M W Greaves (London)
Professor J A Hunter (Edinburgh)

**Speakers:** Professor S T Holgate (Southampton), Professor N Sotor (New York), Professor S Shuster (Newcastle), Dr A Kobza-Black (London), Dr R Champion (Cambridge), Dr J V Griffiths (Janssen UK), Dr R St C Barnetson (Edinburgh), Dr J I Harper (London), Professor R K Chandra (Canada), Dr A Ive (Newcastle)

*Would readers please note that Dr J I Harper will be speaking on 'The Management of Atopic Eczema in Children'. We apologise for the omission of his name from the first announcement of this symposium.*

**Topics**

**Urticaria:** The mast cell; mediators in the pathogenesis of urticaria; pharmacological agents and wealing reactions in the skin; the physical urticarias; clinical aspects of urticaria; astemizole in histamine-mediated skin conditions.

**Atopic eczema:** Immunology; aetiology and management of atopic eczema in children; exclusion diets in pregnant women and incidence of atopic eczema in offspring; management of adult atopic eczema.

*This symposium will be of interest to dermatologists, allergists and physicians with an interest in allergic skin diseases.*

Further information from Dr Jane Griffiths, Janssen Pharmaceutical Limited, Grove, Wantage, Oxon, OX12 0DQ Telephone (02357) 2966.

---

## Methods in Skin Research

Edited by D. and C.J. SKERROW,
Department of Dermatology, University of Glasgow

- An invaluable reference source for all those who study or use the skin

Research into the structure, function and pathology of skin is carried out by clinicians and scientists from an unusually wide range of disciplines. The reasons for this include the size and accessibility of the skin and the wide range of pathological conditions to which it is subject.

This book aims firstly, to promote critical awareness in the field by having a variety of techniques described in detail and in the context of their application to skin research; secondly, to provide scientists and dermatologists wishing to use a technique for the first time with the information which they require in a readily accessible form, and thirdly, to counteract the inappropriate use of techniques by discussing their relative advantages and disadvantages in depth. Particular features are the inclusion of each author's unique "tricks of the trade", discussion of the amount of training needed by a prospective user and the time required to perform experiments. In each case a bibliography of other descriptions and applications is given, together with any experimental minutiae such as the composition of buffers, names of suppliers etc.

Whilst this book will interest all dermatology departments with research facilities, and other workers in the fields of zoology, pathology, cell biology and oncology who study skin or use it as an experimental tissue, it is also hoped that the detailed contributions on the dermatological and more scientific aspects of skin will form a useful bridge between clinicians and scientists and aid the more effective development of the subject.

0471105120 686pp February 1985 £64.00/$112.65

## Papillomaviruses

CIBA FOUNDATION SYMPOSIUM 120

Chairman: P.M. HOWLEY
National Cancer Institute, Bethesda, USA

This book brings together research on molecular genetics and clinical topics and explores the nature of the virus-host cell interaction. Many aspects of papillomavirus biology are covered, from genomic organization and transforming functions to the pathological results and diagnosis of infection. The association of papillomaviruses with epidermodysplasia verruciformis, cervical carcinoma and laryngeal papillomatosis is discussed, as are prospects for therapy and immunization.

0471998370 257pp April 1986 £27.50/$47.50

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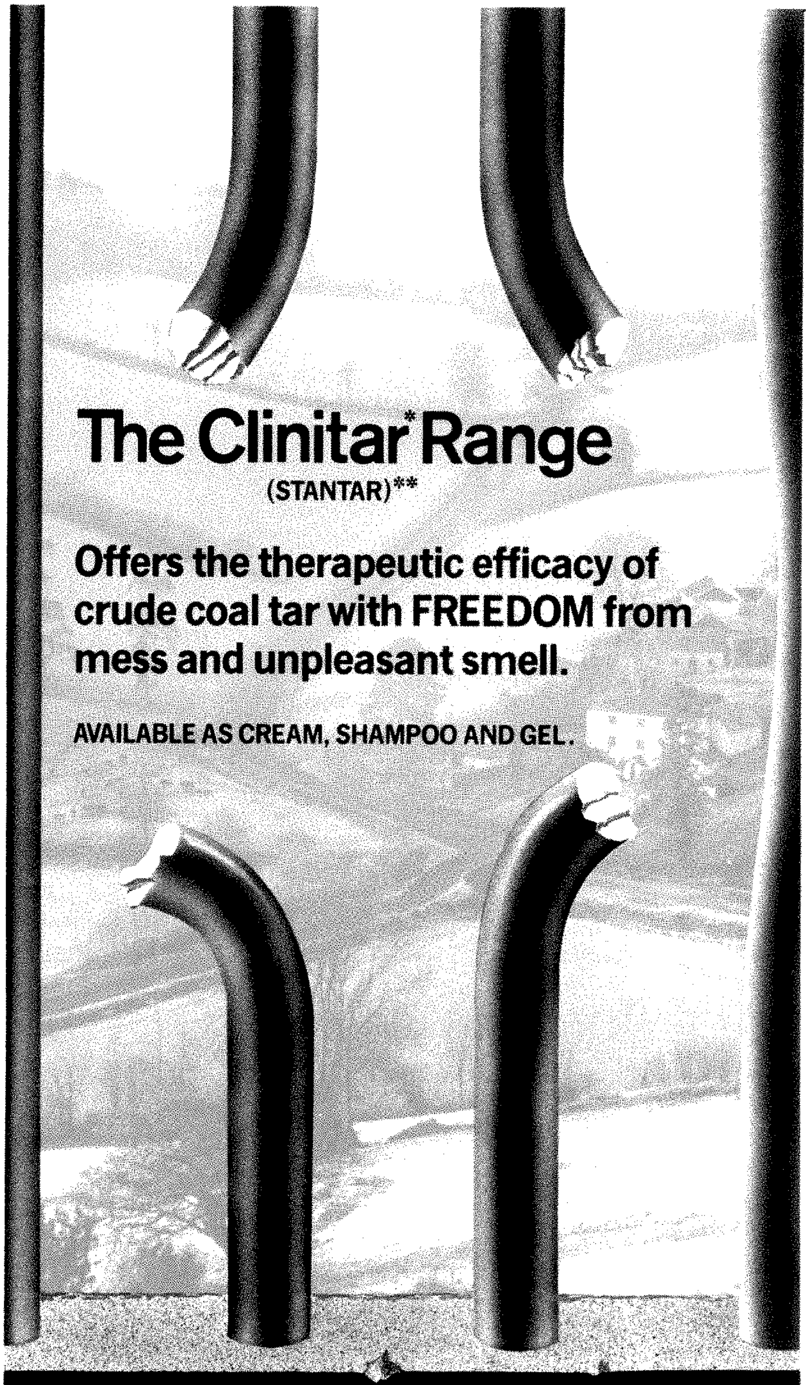

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(STANTAR)**

**Offers the therapeutic efficacy of crude coal tar with FREEDOM from mess and unpleasant smell.**

**AVAILABLE AS CREAM, SHAMPOO AND GEL.**

Further information on Clinitar Cream, Shampoo and Gel are available on request from:

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PRESCRIBING INFORMATION

## **Clinitar** Cream

**PRESENTATION** Greenish-brown oil in water cream containing 1% Stantar
**USES** For the treatment of sub-acute and chronic psoriasis and eczema
**DOSAGE AND ADMINISTRATION** Apply to the affected areas once or twice daily
**CONTRA-INDICATIONS AND WARNINGS** The use of Clinitar cream is not recommended in generalised pustular psoriasis, infections of the skin, or for patients allergic to coal tar. Coal tar can sensitise the skin to U.V. light, leading to sunburn. Clinitar cream should therefore be used cautiously in the sun. Before any irradiation treatment, Clinitar cream should be removed completely
**PHARMACEUTICAL PRECAUTIONS** None
**LEGAL CATEGORY** P
**PACKAGE QUANTITIES** 60g tube
**FURTHER INFORMATION** Clinitar cream contains Stantar, which is an extract of coal tar standardised to have a photosensitising activity corresponding to that of crude coal tar. By using Stantar instead of crude coal tar a product is obtained which is easy to apply, has no unpleasant smell, and is cosmetically inconspicuous
**PRODUCT LICENCE NUMBER** 0033/0110
**BASIC NHS Cost** £2.73

## **Clinitar** Shampoo

**PRESENTATION** A clear brown shampoo with a slightly greenish fluorescence containing 2% Stantar
**USES** For the treatment of pityriasis simplex capitis (dandruff), seborrhoeic dermatitis of the scalp, and psoriasis of the scalp
**DOSAGE AND ADMINISTRATION** Moisten the hair and rub a little shampoo into the hair and scalp. Rinse and repeat the treatment, this time producing a heavy lather. Leave the lather in the hair for 5 minutes after the second treatment and then rinse carefully. Use 1-3 times a week
**CONTRA-INDICATIONS AND WARNINGS** The use of Clinitar shampoo is not recommended for patients allergic to coal tar. Avoid getting shampoo into the eyes
**PHARMACEUTICAL PRECAUTIONS** None. Store at room temperature
**LEGAL CATEGORY** P
**PACKAGE QUANTITIES** 60g tube
**FURTHER INFORMATION** Clinitar shampoo contains Stantar, which is an extract of coal tar standardised to have a photosensitising activity corresponding to that of crude coal tar. By using Stantar instead of crude coal tar a product is obtained which is easier to rub in and wash out and has a pleasant smell
**PRODUCT LICENCE NUMBER** 0033/0109
**BASIC NHS Cost** £2.04

## **Clinitar** Gel

**PRESENTATION** A clear brown gel with a slightly greenish fluorescence, containing Stantar 2.5%
**USES** For the treatment of psoriasis of the scalp and of the body. Other dermatoses responding to tar treatment, such as seborrhoeic dermatitis and chronic eczema
**DOSAGE AND ADMINISTRATION** Apply to the affected areas once or twice daily
**CONTRA-INDICATIONS AND WARNINGS** The use of Clinitar gel is not recommended in generalised pustular psoriasis, infections of the skin, or for patients allergic to coal tar. Coal tar can sensitise the skin to U.V. light, leading to sunburn. Clinitar gel should therefore be used cautiously in the sun. Before any irradiation treatment Clinitar gel should be removed completely. During the summer it is advisable to apply Clinitar gel only once a day at bedtime followed by a bath the next morning. Simultaneous treatments with a coal tar product and drugs which may provoke photosensitivity may create a potential risk for the patient to acquire unintended sunburn on exposure to the sun or sunlight from an artificial source
**PHARMACEUTICAL PRECAUTIONS** None
**LEGAL CATEGORY** P
**PACKAGE QUANTITIES** 40g tube
**FURTHER INFORMATION** Clinitar gel contains Stantar, which is an extract of coal tar standardised to have a photosensitising activity corresponding to that of crude coal tar. By using Stantar instead of crude coal tar a product is obtained which is easy to apply, has no unpleasant smell, and is cosmetically inconspicuous
**PRODUCT LICENCE NUMBER** 0033/0111
**BASIC NHS Cost** £2.59

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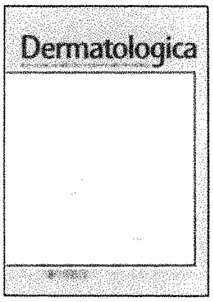

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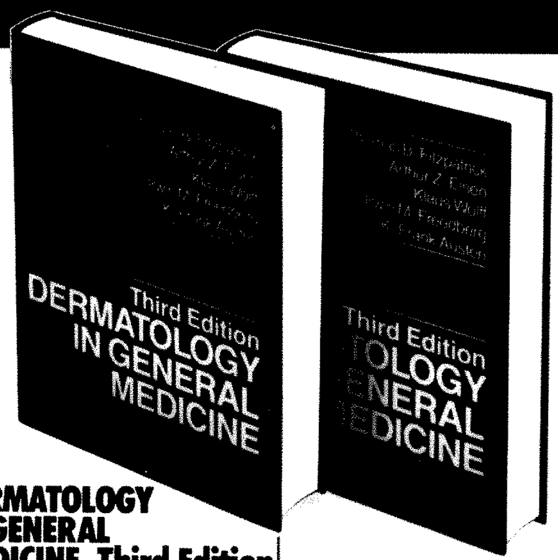

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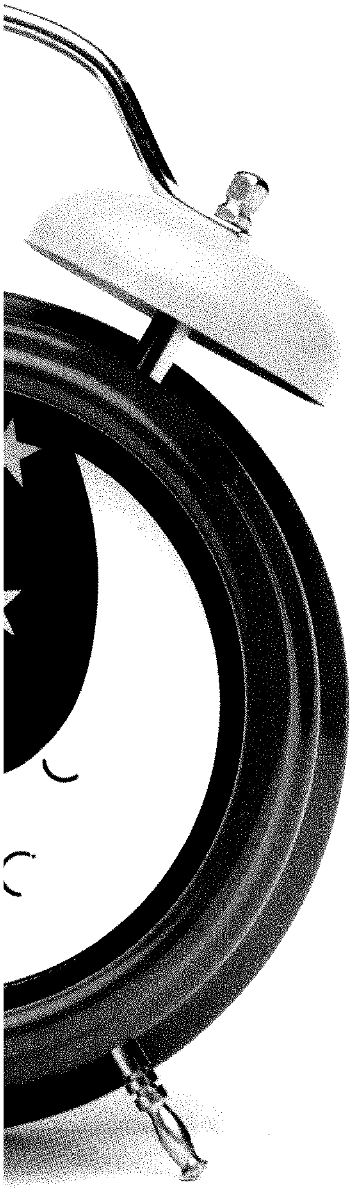

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***Invited Editorial***

# Do epidermotropic T cells exist in normal human skin? A re-evaluation of the SALT hypothesis

S.M.BREATHNACH

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Advances in the past decade have demonstrated that normal mammalian epidermis functions as the most peripheral outpost of the immune system, comprising bone marrow-derived antigen presenting Langerhans cells (Stingl, Tamaki & Katz, 1980; Rowden, 1981), and keratinocytes which secrete a variety of immunoregulatory cytokines. These cytokines include epidermal cell-derived thymocyte activating factor (ETAF), which is indistinguishable from interleukin 1 (IL-1), a cytokine which is indistinguishable from lymphocyte-derived interleukin 3 (IL-3), and a cytokine which augments natural killer cell activity, in addition to prostaglandin E2, leukotrienes and gamma interferon (Sauder, 1984; Luger *et al.*, 1985). ETAF/IL-1 promotes T cell proliferation and differentiation by triggering helper T cell production of interleukin 2 (T cell growth factor), and has been shown recently to be a potent chemotactic agent for polymorphonuclear, mononuclear and T cells (Luger *et al.*, 1983; Sauder, Monick & Hunninghake, 1985). IL-3 promotes the growth of cells of haematopoietic lineage; cultured murine epidermal cells induce bone marrow stem cells to express T lymphocyte differentiation markers (Rubenfield *et al.*, 1981). Keratinocytes contain substances which cross-react immunochemically with serum thymic factor (Kato *et al.*, 1981) and thymopoietin (Chu *et al.*, 1983). The structural and antigenic similarities between the thymus and the epidermis, and the more recent data about keratinocyte-derived immunoregulatory cytokines, have led to renewed speculation that the epidermis may provide a unique inductive micro-environment for post-thymic T cell differentiation. It has been postulated that normal individuals possess sub-populations of epidermotropic T cells which preferentially home to, and recirculate through, the skin; neoplastic T helper cells which infiltrate the epidermis in lesions of cutaneous T cell lymphoma may represent the malignant counterpart of such cells (Patterson & Edelson, 1978; Streilein, 1978). Langerhans cells, keratinocytes, epidermotropic T cells and draining peripheral lymph nodes are thought to form collectively an integrated system of 'skin-associated lymphoid tissues (SALT)' which mediates cutaneous immunosurveillance (Streilein, 1983; 1985).

Central to our present concept of the skin as an organ responsible for immunosurveillance against noxious allergens in the external environment, as embodied in the SALT hypothesis, is the tenet that epidermotropic T cells travel constantly through and sample the epidermis on their path from the circulation to the regional lymph nodes and back. If this is the case, it should be possible to demonstrate readily significant numbers of T cells within normal epidermis. It has been reported that lymphocytes constitute 1–4% of the cells in the basal layer of normal human skin (Andrew & Andrew, 1949). However, it is distinctly unusual to find lymphocytes within the epidermis on ultrastructural examination of normal human skin (A.S.Breathnach, personal communication). The recent identification of a subset of dendritic Thy-1 antigen-bearing, bone marrow-derived epidermal cells, distinct from but present in equivalent numbers to Langerhans cells, in murine epidermis (Tschachler *et al.*, 1983; Bergstresser *et al.*, 1983; Breathnach & Katz, 1984) therefore excited considerable interest, since Thy-1 antigen in the mouse is a marker for peripheral T lymphocytes. Thy-1+ dendritic epidermal cells may develop expression of the helper T cell marker L3T4 in culture (Caughman, Breathnach & Katz, 1985). However, Thy-1+ dendritic epidermal cells do not express the conventional murine T cell markers Lyt 1, 2 or 3. Although they express the natural killer cell marker asialo $GM_1$ (Romani *et al.*, 1985), we have been unable to demonstrate any natural killer cell function of Thy-1+ dendritic epidermal cells (S.M.Breathnach, S.W.Caughman & S.I.Katz, unpublished), and their nature and function remain unknown.

Two obvious questions relating to the SALT hypothesis remain to be answered: firstly, is there a human analogue of the murine Thy-1+ dendritic epidermal cell, and secondly, is it possible, using current immunohistochemical technology, to demonstrate a significant population of intra-epidermal T cells in normal human skin? We, like others, have been unable to identify a population of Thy-1+ human epidermal cells in immunofluorescence studies using both anti-murine and anti-human Thy-1 antibodies (Sontheimer & Seeger 1984; Chambers, Cohen & Crawford, 1985; Cooper *et al.*, 1985). However, Thy-1 antigen in the human, unlike in the mouse, is not present on peripheral blood leukocytes, and negative staining with anti-Thy-1 antibody alone does not exclude the possible existence of a human analogue. Utilizing a battery of anti-leukocyte reagents including anti-asialo $GM_1$ in immunohistochemical, flow cytometric and cell sorting studies, we failed to detect significant numbers of T cells, B cells, monocytes/macrophages other than Langerhans cells, or natural killer cells in tissue sections, epidermal sheets, and epidermal cell suspensions enriched for leukocyte sub-populations by Ficoll-Hypaque density gradient centrifugation (Cooper *et al.*, 1985). Double fluorescence staining with HLe-1, which identifies all human leukocytes, and anti-HLA-DR, which identifies epidermal Langerhans cells, demonstrated that only 0·06% of unfractionated epidermal cells were of the phenotype HLe-1+DR− (i.e. non-Langerhans cells expressing a bone marrow derivation marker). Ultrastructural examination of this apparent sub-population revealed mainly dark basal keratinocytes, which may have non-specifically stained with HLe-1. It is accordingly unlikely that an analogue of the murine Thy-1+ dendritic epidermal cell exists in human epidermis. Moreover, the mean number of cells expressing T cell markers in the epidermis, as assessed by fluorescence microscopy of epidermal sheets, was only 0·25 per $mm^2$, compared with 608 Langerhans cells per $mm^2$; T cells constituted only 0·16% of all epidermal cells in suspension as determined by flow cytometric analysis, even when the suspensions were substantially enriched for mononuclear cell sub-populations by density gradient centrifugation. These findings call into question the occurrence of a significant degree of T cell epidermotropism in normal adult human skin.

What are the implications of these findings for the SALT hypothesis? If there were rapid transit of low numbers of intra-epidermal T cells, then these cells might perhaps be of biological significance. However, it seems more likely that in non-inflamed skin, antigen presentation takes place mainly in the dermis and draining lymph nodes as a result of the migration of antigen-bearing Langerhans cells (Silberberg-Sinakin *et al.*, 1976). The status of the epidermotropic T cell in mycosis fungoides as a malignant version of a normal epidermotropic T cell may also require some rethinking. What mechanisms, then, are available to boost T cell trafficking through the skin in cutaneous inflammatory disorders? Enhanced secretion of ETAF following noxious stimuli applied to the epidermis might lead to T cell chemotaxis towards the skin (Sauder & Katz, 1982; Sauder, 1984; Luger *et al.*, 1985); this could involve site-specific receptors on lymphocytes and complementary tissue-specific structures on dermal high endothelial venules (Butcher, Scollay & Weissman, 1980). The great majority of cells in such dermal inflammatory infiltrates would have been recruited non-specifically. Mechanisms also exist for the preferential recruitment of antigen-specific T cells from the circulation to sites of challenge with specific contact allergens (Scheper, van Dinther-Jannssen & Polak, 1985). Small numbers of antigen-specific T cells may be retained in previous sites of contact sensitivity for prolonged periods, resulting in local specific immunological memory which may be important in retest or flare-up reactivity (Scheper *et al.*, 1983). Keratinocytes have been reported to express Ia/HLA-DR (immune response-associated) antigen in a variety of lymphocyte-mediated skin diseases, presumably as a result of gamma interferon secretion by activated lymphocytes (reviewed in Breathnach & Katz, 1986). Ia/HLA-DR positive keratinocytes may in turn enhance lymphocyte and Langerhans cell entry into the skin (Daynes *et al.*, 1985). It has been suggested that ETAF-secreting Ia/HLA-DR positive keratinocytes may be able to present antigen directly to intra-epidermal T cells and thus greatly enhance cutaneous immune responses. Current research is being directed at investigating the potential immunological function of Ia/HLA-DR positive keratinocytes, and at determining whether ETAF and/or other T cell chemotactic factors are indeed secreted in increased amounts in cutaneous inflammatory diseases.

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# Scl 70 antibody—a specific marker of systemic sclerosis

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## SUMMARY

Scl 70 antibodies were tested for in 107 patients with systemic sclerosis: 68 with acrosclerosis and 39 with diffuse scleroderma. Anticentromere antibodies (ACA) and other antinuclear antibodies (ANA) were tested for by indirect immunofluorescence on HEp-2 cells. Positive results for Scl 70 antibodies were obtained in 77% of cases of diffuse scleroderma and 44% of acrosclerosis. ACA and Scl 70 antibodies were found to be mutually exclusive. If acrosclerosis cases positive for anticentromere antibodies are excluded, the percentage of acrosclerosis cases positive for Scl 70 was 63%. ACA were found to be a marker of a benign, abortive subset of acrosclerosis with almost no cutaneous involvement (CREST), whereas Scl 70 did not discriminate between acrosclerosis and diffuse scleroderma. On HEp-2 cells Scl 70 positive sera gave a characteristic, fine speckled, almost homogeneous nuclear staining pattern.

Antinuclear antibodies (ANA) are detectable in the majority of cases of systemic sclerosis (Jordon *et al.*, 1977; Tan *et al.*, 1980). In our series of 298 cases followed up for several (usually more than 10) years, the frequency of ANA-positive tests on tissue substrates in repeated studies ranged from 80% in acrosclerosis to 94% in diffuse scleroderma (Jablonska *et al.*, 1986). In a proportion of acrosclerosis cases with negative ANA findings on tissue subtrates, the test performed on the human cell line HEp-2 disclosed anticentromere antibodies (ACA) (Chorzelski *et al.*, 1985). These are considered to be a marker of CREST (Tan *et al.*, 1980; Moroi *et al.*, 1980; Fritzler, Kinsella & Garbutt, 1980; Kallenberg *et al.*, 1982; Cattogio *et al.*, 1983; Tuffanelli *et al.*, 1983; Steen *et al.*, 1984; Chorzelski *et al.*, 1985). In our series, ACA were invariably absent in patients with diffuse scleroderma.

Another antibody, Scl 70, detected by immunodiffusion, has been found mainly in patients with diffuse scleroderma (Tan *et al.*, 1980). Scl 70 antigen, prepared from rabbit thymus, is a basic non-histone protein located on the chromosomes of the cells in mitosis (Douvas, Achten & Tan, 1979). In patients with diffuse scleroderma, antibodies to Scl 70 were found in 23% (6 of

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26) by Tan *et al.* (1980) and in 34% (10 of 29) by Cattogio *et al.* (1983). In CREST (acrosclerosis) Scl 70 antibodies were reported in 13% of patients: 3 of 24 patients reported by Tan *et al.* (1980) and 6 of 46 patients reported by Cattogio *et al.* (1983).

The aim of the present study was to evaluate the specificity of Scl 70 antibody for systemic sclerosis compared with that of ACA, and to measure the frequency of occurrence of Scl 70 in acrosclerosis and diffuse scleroderma, and its association with ANA and ACA.

## METHODS

### *Patients*

The studies were performed in 107 patients with systemic sclerosis: 68 with acrosclerosis and 39 with diffuse scleroderma (Table 1). Controls comprised 90 patients with other connective tissue diseases, 10 with mixed connective tissue disease (MCTD), 37 with systemic lupus erythematosus (SLE), 22 with other collagen diseases and 18 with chronic glomerulonephritis (Table 2).

The criteria for diagnosis of acrosclerosis were: a protracted and relatively benign course, limited skin changes (usually confined to the hands, forearms and face, with telangiectases and, frequently, calcium deposits), and pronounced Raynaud's phenomenon preceding indurations and visceral involvement by several years (Jablonska, 1975; Rodnan, Jablonska & Medsger, 1979). Diffuse scleroderma was classified according to the preliminary ARA criteria of Masi *et al.* (1980) and according to Jablonska (1975).

TABLE 1. Frequency of Scl 70 antibody in patients with systemic scleroderma

| Diagnosis | No. of cases | No. positive (%) |
|---|---|---|
| Acrosclerosis | 68 | 30 (44) |
| Diffuse scleroderma | 39 | 30 (77) |
| Total | 107 | 30 (56) |

TABLE 2. Incidence of Scl 70 in controls

| Diagnosis | No. of cases | Scl 70 no. positive |
|---|---|---|
| MCTD | 13 | 2 |
| SLE | 37 | 0 |
| Other collagen diseases* | 22 | 0 |
| Chronic glomerulonephritis without symptoms of collagenoses | 18 | 0 |
| Total | 90 | 2 |

* Dermatomyositis, 3; DLE and DDLE, 4; morphea, 6; suspected collagenoses, 9.

Patients were classified as having diffuse scleroderma if indurations of the skin involved the face, extremities and the trunk ('central cutaneous involvement'), often with symmetrical hyper- and hypopigmentation. The course was usually more rapid and severe, with a short period between the onset of Raynaud's phenomenon and the onset of indurations (Jablonska, 1975; Masi *et al.*, 1980).

Patients were considered to have lung involvement if they had any one of the following findings:

Bibasilar pulmonary interstitial fibrosis on chest X-ray;
vital capacity, forced expiratory volume for first second, mid-expiratory flow, or thoracic gas volume less than 75% of predicted normal.

Patients were considered to have heart involvement if they had any one of the following findings on electrocardiography, ECHO-cardiography and/or X-ray: cardiac arrhythmias, conduction disturbances, right heart failure secondary to pulmonary hypertension, or pericarditis. Patients were considered to have kidney involvement if they had abnormal clearance and/or changes in urinalysis, and were considered to have muscle involvement if they had any one of the following findings: primary muscle involvement on electromyography, increased levels of muscle enzymes: creatine-phosphokinase, aldolase and transaminases, or morphological changes in muscle biopsy.

MCTD was diagnosed according to the clinical and serological criteria of Sharp *et al.* (1979) and SLE according to the revised ARA criteria (Tan *et al.*, 1982).

### *Antibody testing*

Scl 70 antigen was prepared from calf thymus according to the method of Tan *et al.* (1980), with certain modifications by Kumar *et al.* (1984). The antigen is very unstable, and antigenicity is lost if it is stored for prolonged periods at 4°C. 50 g calf thymus freshly obtained from the slaughter house was cut into small cubes and homogenized in a Waring blender with 100 ml of phosphate buffered saline containing 1 mM of mercaptoethanol and 0·1 mM of phenylmethylsulphonyl fluoride. The homogenate was stirred for 2 h at 4°C and then centrifuged at 14 000 ***g*** for 1 h. The supernatant, which contained primarily RNP and Sm activity, was discarded. The pellet was suspended in phosphate buffered saline containing 0·5 M NaCl and mixed for 1 h. The supernatant obtained upon centrifugation of this homogenate (at 68 000 ***g*** for 2 h) contained Scl 70 activity and was extensively dialysed against distilled water and freeze dried. The freeze dried preparation was dissolved in 10 mM Tris-HCl buffer pH 7·5 to give a final protein concentration of 200 mg/ml.

Immunodiffusion was performed according to the method of Tan *et al.* (1980), as standardized for the detection of antibodies to RNP and Sm by Kumar *et al.* (1984).

All sera were also tested on HEp-2 cells for ACA and other ANA, using the method described previously (Beutner *et al.*, 1985). The conjugate used was fluorescein labelled goat anti-human IgG (Heintel, Vienna, Austria), with a fluorescein to protein ratio of 2·2, 16 units/ml. This was diluted 1:128 to contain $\frac{1}{8}$ units/ml.

## RESULTS

Scl 70 antibody appeared significantly more frequently in diffuse scleroderma (77% of patients) than in acrosclerosis (44%) ($P < 0{\cdot}05$; Table 1). The controls were negative, with the exception of two patients with MCTD (Table 2), both falling within the spectrum of systemic sclerosis (pronounced Raynaud's phenomenon with indurative oedema of the hands, mask-like facies with thinning of the lips and, in one, diminished oesophageal motility). Thus, Scl 70 appears to be highly specific of systemic sclerosis. Patients with acrosclerosis positive for ACA were invariably negative for Scl 70 (Table 3). Conversely, patients with acrosclerosis negative for ACA were found to be positive for Scl 70 in 63% cases, whereas in acrosclerosis patients as a whole Scl 70 antibody was present in 44% of the cases (Table 1).

It should be emphasized that among 39 cases of diffuse scleroderma, 16 observed by us for more than 10 years were classified initially as severe acrosclerosis or transitional form, acrosclerosis-diffuse scleroderma. The frequency of Scl 70 antibody in this group was 12 of 16 (75%), i.e. essentially the same as in typical cases of diffuse scleroderma. Therefore, the transitional form was grouped with diffuse scleroderma.

Immunofluorescence studies on HEp-2 showed a characteristic pattern with all sera positive in the immunodiffusion test for Scl 70 antibody. The fluorescence pattern was almost homogeneous due to diffuse fine, densely packed speckles associated, in most cases, with weak nucleolar staining (Fig. 1).

If patients are classified according to the varieties of systemic sclerosis and presence or absence of Scl 70 antibody, slight differences can be seen (Tables 4 and 5). There were no

TABLE 3. Occurrence of Scl 70 antibody with anticentromere antibody in patients with acrosclerosis

| Diagnosis | No. of cases | Scl 70 no. positive (%) |
|---|---|---|
| ACA-negative | 48 | 30 (63) |
| ACA-positive | 20 | 0 |

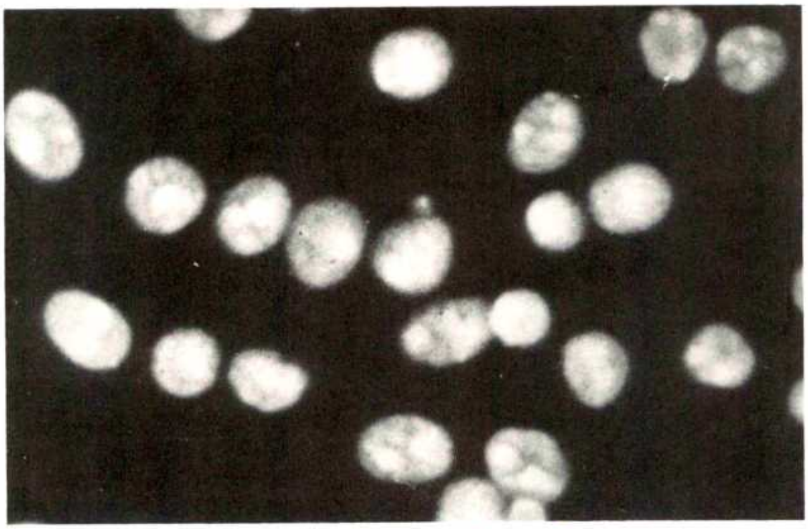

FIGURE 1. Immunofluorescence pattern of Scl 70 antibody on HEp-2 cells: fine granular, almost homogeneous staining with visible nucleoli (original × 400).

TABLE 4. Clinical and laboratory findings in Scl 70 positive and Scl 70 negative patients with acrosclerosis

| Clinical and laboratory findings | Scl 70 positive (30 cases) | Scl 70 negative[1] (38 cases) | Scl 70 negative ACA-negative[2] (18 cases) |
|---|---|---|---|
| Mean age (years) | 49·8 | 47·5 | 44·8 |
| Sex ratio F:M | 28:2 | 34:4 | 17.1 |
| Mean duration of Raynauds phenomenon (years) | 18·3 | 12·3 | 13·2 |
| Mean duration of sclerosis (years) | 11·4 | 5·2 | 6·2 |
| Mean period between appearance of Raynauds phenomenon and indurations (years) | 6·9 | 6·2 | 7·0 |
| Indurative oedema and/or indurations confined to the digits | 18/28 (64%) | 32/36 (89%)* | 14 (82%) |
| Sclerodactyly with contractures and immobilization of fingers ('grip') | 12/28 (43%) | 5/36 (14%)** | 5/17 (29%) |
| Digital scars and/or acrolysis | 23/28 (82%) | 22/36 (61%) | 10/17 (56%) |
| Cutaneous involvement extending up to one third of forearm | 11/28 (39%) | 7/36 (9%) | 7/17 (41%) |
| Calcinosis | 6/28 (21%) | 17/36 (47%)* | 8/17 (47%) |
| Telangiectasia | 22/28 (78%) | 30/36 (83%) | 14/17 (82%) |
| Central cutaneous involvement[3] | 6/28 (21%) | 0/36** | 0/17* |
| Arthralgia | 24/28 (86%) | 31/36 (86%) | 15/17 (88%) |
| Decreased oesophageal motility | 14/28 (50%) | 22/36 (61%) | 11/17 (65%) |
| Heart involvement | 9/26 (35%) | 24/36 (67%)* | 10/17 (59%) |
| Kidney involvement | 0/28 | 0/36 | 0/17 |
| Lung involvement | 13/28 (46%) | 15/36 (42%) | 6/17 (35%) |
| Muscle involvement and/or EMG abnormalities | 3/28 (11%) | 7/36 (31%) | 4/17 (24%) |
| *Immunological findings* | | | |
| ANA other than ACA—on HEp-2 | 23/23 (100%) | 9/18 (50%)** | 9/18 (50%)** |
| Positive at a titre ≥ 1:80—on monkey oesophagus | 19/30 (63%) | 10/38 (26%)** | 9/18 (50%) |
| Exclusively nucleolar pattern | 0/30 | 3/38 (8%) | 3/18 (17%)* |
| RNP positive | 0/30 | 4/38 (11%) | 4/18 (22%)* |

[1] This group includes ACA-positive and ACA-negative cases.

[2] In this group only cases negative for ACA are included; cases positive for ACA have been described in detail previously (Chorzelski *et al.*, 1985).

[3] Some indurations and/or atrophy, mainly on the thorax.

Significantly different from SCL 70 positive patients (fraction test) $^{*}P < 0·05$, $^{**}P < 0·01$.

differences in major clinical characteristics, i.e. age, sex, and time between appearance of Raynaud's phenomenon and skin indurations, between the SCL 70 positive and negative groups.

As can be seen in Table 4, skin indurations confined to the digits, and calcinosis were more frequent in acrosclerosis cases negative for Scl 70, whereas immobilization and contractures of the fingers were more frequent in patients positive for Scl 70. However, if only cases negative for ACA are evaluated, these differences were not statistically significant. Visceral involvement was comparable in the two groups, with the exception of somewhat more frequent heart changes in Scl 70 negative cases. It should be stressed that central cutaneous involvement was invariably absent in patients negative for Scl 70, whereas slight changes on the thorax were noticed in 20% of cases positive for Scl 70.

TABLE 5. Clinical and laboratory findings in Scl 70 positive and Scl 70 negative cases with diffuse scleroderma

| Clinical and laboratory findings | Scl 70 positive (30 cases) | Scl 70 negative (9 cases) |
|---|---|---|
| Mean age (years) | 45·3 | 39·7 |
| Sex ratio F:M | 27·3 | 8:1 |
| Mean duration of Raynauds phenomenon (years) | 7·8 | 7·4 |
| Mean duration of sclerosis (years) | 3·9 | 4·0 |
| Mean period between appearance of Raynauds phenomenon and indurations (years) | 3·9 | 3·4 |
| Indurative oedema and/or indurations confined to the digits | 19/27 (70%) | 7/9 (78%) |
| Sclerodactyly with contractures and immobilization of fingers ('grip') | 14/27 (52%) | 2/9 (23%) |
| Digital scars and/or acrolysis | 26/27 (96%) | 5/9 (55%)** |
| Cutaneous involvement extending up to one third of the forearm | 25/27 (93%) | 8/9 (89%) |
| Calcinosis | 12/27 (44%) | 1/8 (13%) |
| Telangiectasia | 17/27 (63%) | 1/9 (11%)* |
| Central cutaneous involvement | 26/27 (96%) | 7/9 (78%) |
| Arthralgia | 27/27 (100%) | 8/9 (89%) |
| Decreased oesophageal motility | 20/27 (74%) | 4/9 (44%) |
| Heart involvement | 19/27 (70%) | 8/9 (89%) |
| Lung involvement | 21/27 (78%) | 2/9 (22%)** |
| Kidney involvement | 7/27 (26%) | 2/9 (22%) |
| Muscle involvement and/or EMG abnormalities | 11/27 (41%) | 5/9 (56%) |
| ANA-positive at a titre $\geqslant$ 1:80—on HEp-2 | 25/25 (100%) | 5/8 (63%)** |
| —on monkey oesophagus | 27/30 (90%) | 5/9 (55%)* |
| Nucleolar pattern | 0/30 | 3/9 (33%)** |
| RNP positive | 0/30 | 1/9 (11%) |

Significantly different from Scl 70 positive group *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ compared using a fraction test.

As can be seen in Table 5, digital scars and acrolysis, telangiectases and lung involvement were more frequent in cases of diffuse scleroderma positive for Scl 70.

Antinuclear antibodies, as detected on HEp-2 cells and monkey oesophagus, were found in a higher percentage of cases of acrosclerosis and diffuse scleroderma positive for Scl 70 (Tables 4 and 5).

RNP antibodies were found in five cases negative for Scl 70, four cases of acrosclerosis and one of diffuse scleroderma. Nucleolar staining without any associated patterns was found in five cases, four of acrosclerosis and one of diffuse scleroderma, all negative for Scl 70.

## DISCUSSION

Scl 70 antibody was found to be a specific serological marker of systemic scleroderma. In our series it was detected at a much higher frequency than reported in previous publications (Tan *et al.*, 1980; Catoggio *et al.*, 1983), but comparable results have been reported recently by Takehara, Moroi and Ishibashi (1985). The higher detection rates of Scl 70 in our study, 77% in diffuse scleroderma and 44% in acrosclerosis, may be related to differences in the type of cases studied and, at least in part, to the technique of antigen preparation (Kumar *et al.*, unpublished observations).

On HEp-2, sera positive for Scl 70 showed a very characteristic, fine speckled, almost homogeneous immunofluorescence pattern, which could be recognized easily. This pattern corresponds to the fine speckles described by Tan *et al.* (1980). If the titre of ANA with this pattern on HEp-2 was higher than 1:80, it was invariably associated with a positive immunodiffusion test for Scl 70. Since immunodiffusion is less sensitive than immunofluorescence, it is to be expected that Scl 70 antibody, demonstrated in lower titres in the indirect IF test, would not be detectable by immunodiffusion. Thus, it is conceivable that Scl 70 antibody occurs even more frequently than detected in our study.

In contrast to ACA antibody, which is a marker of an abortive subset of acrosclerosis with almost no cutaneous indurations (Chorzelski *et al.*, 1985), Scl 70 is a marker of systemic sclerosis as a whole, with prevalence in diffuse scleroderma, but present also in a high proportion of cases of acrosclerosis. Thus, it does not discriminate between the subsets of systemic scleroderma. There were only slight clinical differences between cases positive and negative for Scl 70. In patients with acrosclerosis negative for Scl 70, the indurations were confined more frequently to the fingers and there were fewer contractures of the fingers; calcinosis was found in a somewhat larger proportion of the patients, and there was no induration or atrophy on the trunk; the disease was thus less severe than in patients positive for Scl 70 antibody.

In patients with diffuse scleroderma the clinical differences between patients positive and those negative for Scl 70 were less conspicuous.

Antinuclear antibodies were found at titres above 1:80 on HEp-2 in all patients positive for Scl 70. They were significantly less frequent in both acrosclerosis and diffuse scleroderma patients negative for Scl 70. On monkey oesophagus, ANA at titres above 1:80 were not found in all Scl 70 positive cases, but they also occurred significantly more frequently in acrosclerosis and diffuse scleroderma cases positive for Scl 70 (Table 5).

ACA positive cases of acrosclerosis were, in our study, invariably negative for Scl 70 antibody. Only two exceptional cases positive for Scl 70 and ACA antibodies have been reported by Ruffatti *et al.* (1985), and the authors stress that this is a rare finding.

Scl 70 was not detected in other collagen diseases, with the exception of two overlap cases, both with some cutaneous symptoms of scleroderma (MCTD within the spectrum of scleroderma).

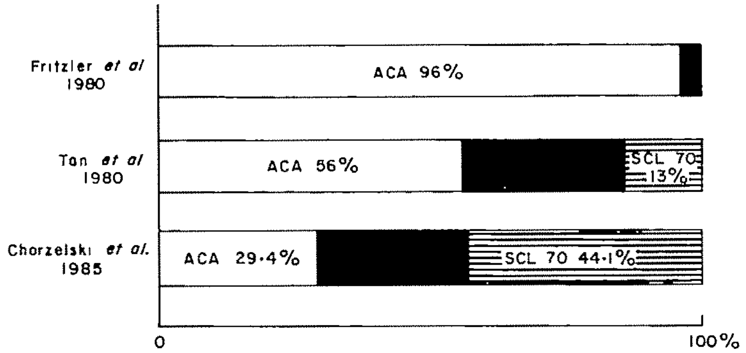

FIGURE 2. Published incidence of ACA and Scl 70 in acrosclerosis. □. Percentage of patients positive for ACA, and ■: for SCL 70. ■: Percentage of patients negative for both ACA and SCL 70.

As shown in Figure 2, the gap between the frequency of ACA and of Scl 70 will probably be narrowed in future studies either by increasing the sensitivity of the methods or by detection of other, as yet unidentified antibodies. In our series, this gap is filled, in a part, by the RNP and anti-nucleolar antibodies.

The value of tests for Scl 70 antibody and ACA may well prove to lie also in their prognostic role, in predicting whether patients with acrosclerosis will, in time, develop into true diffuse scleroderma or remain as cases of acrosclerosis with minimal cutaneous involvement. This is supported by more than 10 years of retrospective study on 16 cases of diffuse scleroderma, diagnosed originally as acrosclerosis, and later as transitional form; 12 of these 16 (75%) were positive for Scl 70 antibody. Follow-up studies are needed to determine whether cases of acrosclerosis that are positive for Scl 70 antibodies are at greater risk of developing frank diffuse scleroderma.

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# Degradation of porcine dermal connective tissue by collagenase and hyaluronidase

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## SUMMARY

Selective destruction of connective tissue may be a useful therapeutic tool in conditions associated with abnormal deposition of scar tissue. We have investigated intradermal injections of clostridial collagenase and bovine testicular hyaluronidase alone and in combination in Yucatan miniature hairless pigs. Collagenase in combination with hyaluronidase was quite efficient at destroying the connective tissue matrix, although elastic tissue appeared to be completely spared. Collagenase alone at higher doses degraded collagen, but hyaluronidase had little effect on connective tissue architecture.

The extracellular matrix within the dermis is a dense meshwork of collagen and elastin, embedded in a ground substance composed of glycoproteins and proteoglycans. Collagenases are enzymes which have a crucial role in the degradation of tissue collagen (Evanson, 1974), a process essential in connective tissue growth and remodelling. Hyaluronidases are enzymes which play a role in the degradation of mucopolysaccharides composing the ground substance of the dermis.

Skin diseases resulting from abnormal uncontrolled growths of connective tissue, such as keloids, hypertrophic scars and connective tissue naevi, are often a vexing treatment problem. Since collagenase and hyaluronidase are enzymes capable of degrading this extracellular matrix, they may have the potential to alter favourably the abnormal connective tissue in these diseases.

In this study, we examined the effect of intradermal administration of clostridial collagenase and bovine testicular hyaluronidase, used singly and in combination, in an animal model, in order to gain a better understanding of their action on normal dermis. The Yucatan miniature pig was used because, in addition to its small size, its skin resembles human skin in basic architecture more closely than does the skin of any of the other common small laboratory

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animals. The structure and amount of dermal collagen and elastic tissue is similar to that of human skin.

## METHODS

Yucatan miniature hairless pigs (Charles River Laboratories, Wilmington, MA, U.S.A.) were used for injections.

Clostridial collagenase (Nucleolysin, lyophilized, Advanced Biofactures, Lynbrook, NY, U.S.A.) was dissolved in 0·15 M NaCl, 0·1 M $CaCl_2$. Bovine testicular hyaluronidase (Wydase, lyophilized, Wyeth, Philadelphia, PA, U.S.A.) was dissolved in 0·15 M NaCl, 0·1 M $CaCl_2$. Collagenase was used alone at concentrations of 50, 100, 200, 400, 500 and 600 units. Hyaluronidase was used alone at concentrations of 15, 45 and 150 units. Collagenase at concentrations of 200, 400, 500 and 600 units was used in combination with either 15, 45 or 150 units of hyaluronidase. Injections of 0·1 ml were given intracutaneously.

### *Skin biopsies*

6 mm punch biopsy specimens were obtained from the treatment sites at 1, 2, 7 and 14 days following injection and were compared with biopsies from untreated control sites on the same animal. The biopsies were fixed in 10% buffered formalin for 48 h and processed routinely. Multiple 5 μm thick step sections of each block were obtained and stained using haematoxylin and eosin. In each case, Masson's trichome stain was employed to study the tinctorial properties of the dermal collagen, Verhoeff-van Gieson stain was used to evaluate the dermal elastic fibres and colloidal iron stain was used to evaluate dermal glycosaminoglycans.

Using routine light microscopy, the following were evaluated: area of dermis showing degenerative changes, extent of collagen degeneration, degree of inflammatory response and wound healing response, seen as fibroblast and capillary bud proliferation with new collagen formation and increased deposition of glycosaminoglycans.

## RESULTS

### *Gross examination*

There was no evidence of systemic upset in the animals. Palpation of the injection sites did not appear painful. Other than occasional haemorrhage about the collagenase and collagenase plus hyaluronidase injection sites, the lesions were unremarkable in appearance throughout the experiment.

### *Histological examination*

*Hyaluronidase alone*. At doses of 15 and 150 units a widespread inflammatory infiltrate was seen coursing between collagen bundles and in perivascular connective tissue (Fig. 1). The infiltrate was dominated by polymorphonuclear leuckocytes from one to two days post injection. The infiltrate from one week onwards comprised lymphocytes and numerous eosinophils. No significant collagen degradation was noted. Only mild degenerative changes limited to the periphery of the collagen bundles nearest the inflammatory cells were seen. No decrease in dermal proteoglycan was noted; however, only trace amounts of proteoglycan were demonstrable with colloidal iron stain even in normal untreated control skin. No granulation tissue formation or fibroblast proliferation, as evidence of a wound healing response, was noted.

*Collagenase alone*. Collagenase alone produced an obvious dose-response relationship. At

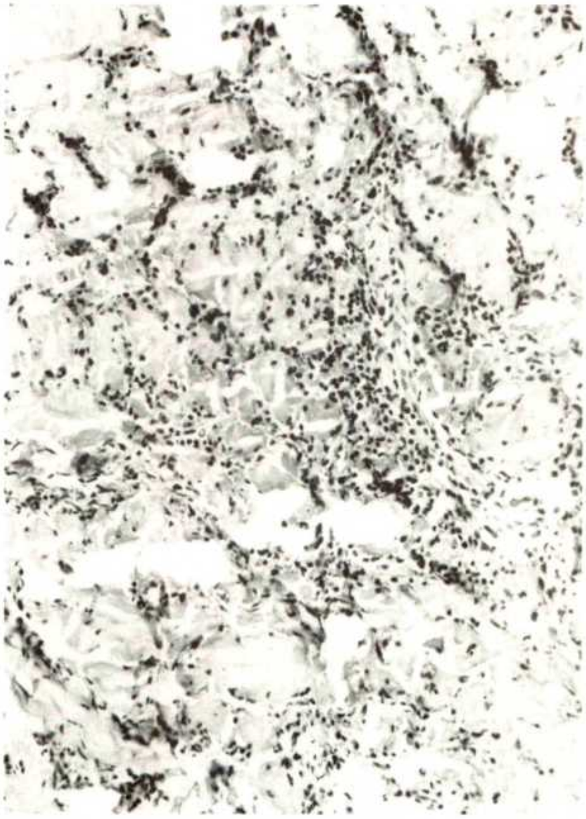

FIGURE 1. Hyaluronidase 150 units, 7 days. Mixed inflammatory infiltrate permeates between collagen bundles which show little or no degenerative change. (Haematoxylin and eosin, original × 170).

doses of 50 to 200 units, no significant changes were seen when compared with untreated controls. With doses of 400 and 500 units, a moderate degree of collagen degeneration was seen within the zone of dermal involvement (Fig. 2). Although numerous collagen bundles showed complete lysis, many remained partially intact. This was accompanied by a moderate inflammatory infiltrate. A prominent accumulation of fibroblasts, capillary buds, new collagen and glycosaminoglycan deposition was present at the injection site at 2 weeks. Elastic fibres were well preserved in and around the zone of degenerated collagen fibres.

*Collagenase and hyaluronidase*. The combinations of hyalurondiase and collagenase produced maximum collagen degeneration. Large, spreading zones of complete collagen degradation and a dense inflammatory response were seen around the injection sites at all dose levels (Fig. 3). Elastic fibres were well preserved in these zones, showing no evidence of degenerative changes (Fig. 4). At 2 weeks, marked fibroblast proliferation accompanied by new dermal collagen and capillary bud formation was seen. Increased formation of glycosaminoglycans was associated with the fibroblast proliferation. This wound healing response was restricted to the zone of previous collagen lysis. Focal epidermal necrosis was seen overlying these zones when the injection sites were relatively superficial within the dermis. The degree of degenerative change was similar with 15 and with 150 units of hyaluronidase. Marked degenerative changes were seen with as little as 200 units of collagenase when combined with 15 units of hyaluronidase. No

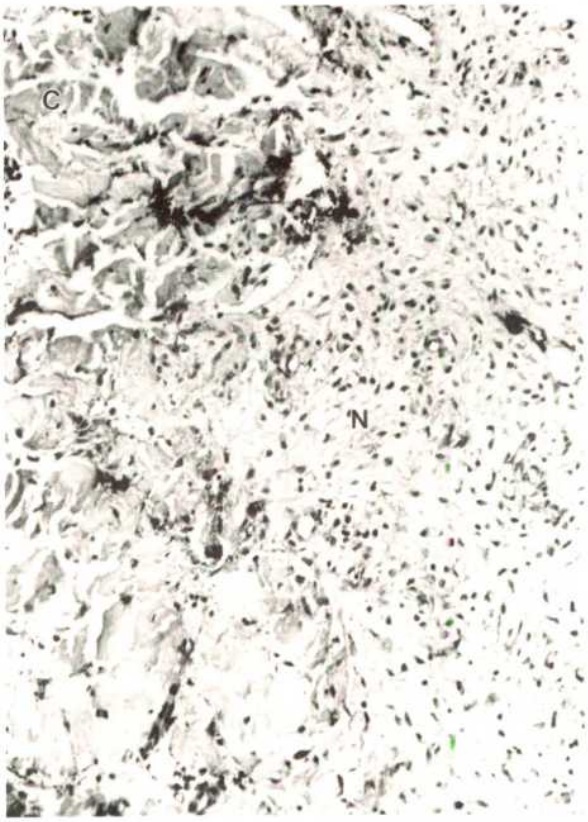

FIGURE 2. Collagenase 500 units, 7 days. Preserved collagen on left (C). Right side of field shows partial to complete necrosis of collagen bundles within zone of involvement around the injection site (N). (Haematoxylin and eosin, original × 170).

dose-response relationship was seen with increasing concentrations of collagenase from 200 to 600 units.

## DISCUSSION

Bacterial collagenase is an enzyme obtained from *Clostridium histolyticum*. It is specific for the unique helical structure of collagen, requiring the amino acid sequence Pro-$X$-Gly-Pro-$Y$, where $X$ and $Y$ can be any amino acid (Siefter & Harper, 1971). The enzyme cleaves the bond between the $X$ and Gly to release a Gly-$NH_2$ terminus (Luebaga-Mukasa, Harper & Taylor, 1976). Hyaluronidases are enzymes occurring in testis, leech extract, snake venom and many bacteria, and have been found in autolysates of skin (Roden, 1967). These enzymes are capable of hydrolysing the carbon-1 of the glucosaminidic bond in hyaluronic acid, chondroitin-4-sulphate and chondroitin-6-sulphate. They do not hydrolyse dermatan sulphate (Linares & Larson, 1978).

In the present study, hyaluronidase alone was unable to degrade collagen bundles to any significant degree. It did, however, provoke a moderate cellular inflammatory response which permeated through a large zone of the extracollagenous connective tissue matrix around the

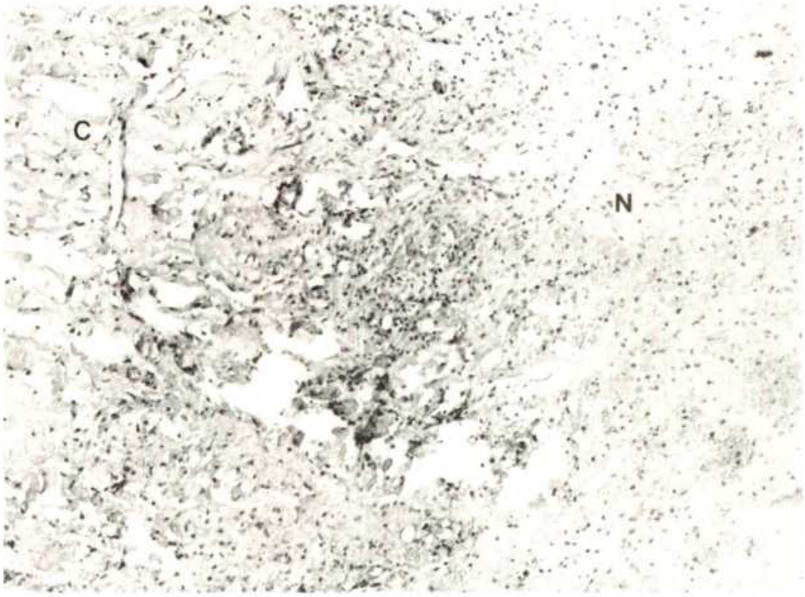

FIGURE 3. Collagenase 500 units and hyaluronidase 150 units, 7 days. Preserved collagen in upper left-hand corner of field (C). Right side of field shows a large zone of complete collagen degradation with a pool of liquefaction necrosis (N). (Haematoxylin and eosin, original × 100).

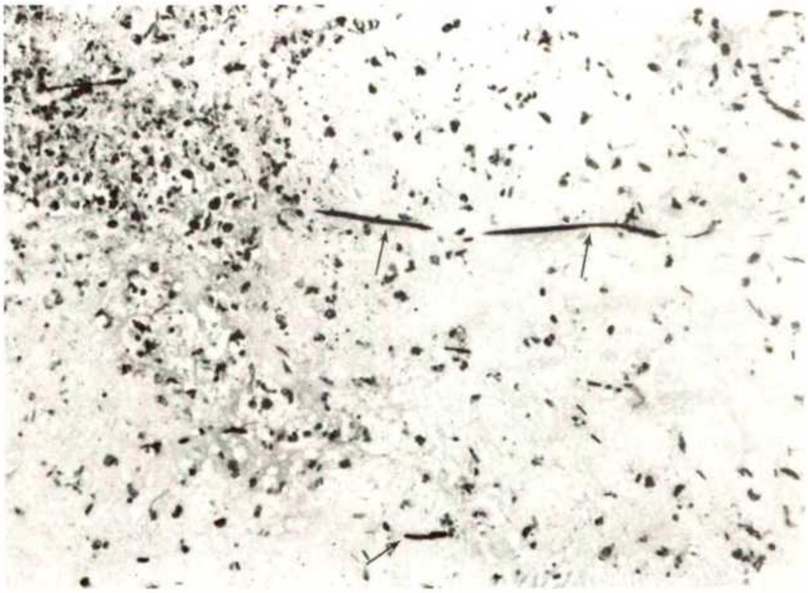

FIGURE 4. Collagenase 500 units and hyaluronidase 150 units, 7 days. Note preservation of elastic fibres (arrows) within zone of complete collagen lysis. (Verhoeff-van Gieson stain, original × 250).

injection site. Collagenase at doses of 400 units or greater was able to cause significant localized collagen degradation at the injection site. The degradation was limited, in that many collagen bundles showed only partial necrosis. Collagenase in combination with as little as 15 units of hyaluronidase caused complete collagen degeneration in a spreading fashion in a large zone around the injection site. All collagen bundles within this zone showed complete lysis. Elastic fibres were preserved in all of the experiments, even in areas of massive connective tissue breakdown.

The combination of collagenase and hyaluronidase is a potent tool for degrading collagen and altering dermal connective tissue in this animal model. Relatively low concentrations of each can be used in combination to produce dramatic degradation of dermal collagen while preserving the elastic fibre framework. Although theoretically possible, there was no evidence of either immediate or delayed hypersensitivity reactions, despite repeated injections in the same animal over long periods of time. These enzymes may prove to be a valuable tool in the treatment of diseases such as keloids, hypertrophic scars and connective tissue naevi, where an altered state of collagen formation is present, leading to excessive deposition of dermal collagen.

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# Immunolocalization of collagenase and tissue inhibitor of metalloproteinases (TIMP) in hypertrophic scar tissue

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## SUMMARY

Normal, mature and hypertrophic dermal scars were examined by indirect immunofluorescence for the presence of collagenase, tissue inhibitor of metalloproteinases (TIMP) and cathepsin D. Significant extracellular immunoprecipitation of both collagenase and TIMP were found in areas of all scars judged to be actively remodelling, whereas inactive areas were predominantly negative. TIMP was also present in endothelial cells of patent blood vessels, but found not to be present in the enlarged endothelial cells of occluded vessels. Negligible amounts of extracellular cathepsin D were found.

Hypertrophic scars can develop as a result of deep dermal injury. They are characterized by the presence of excess collagen, in the form of discrete nodules, and an increased density of blood vessels, most of which are partially or totally occluded due to an excess of endothelial cells (Kischer, Shetlar & Chvapil, 1982). There are few identifiable inflammatory cells. Mammalian collagenases have been implicated in collagen degradation both in normal connective tissue turnover and in pathological conditions (for review, see Woolley, 1984); during wound healing, when re-epithelialization has begun but is not yet complete, remodelling of the dermal collagen is very active and there is a concommitant rise in collagenase activity (Grillo & Gross, 1967; Eisen, 1969). High collagenase activity has been extracted directly from human post-burn granulation tissue (Kishi *et al.*, 1984). Collagenase activity was also measured in hypertrophic scars and was found to be higher than in normal skin, where virtually no activity was seen (Cohen, Diegelmann & Keiser, 1976). However, the cellular source of the collagenase activity within hypertrophic scars has not yet been established.

The tissue inhibitor of metalloproteinases (TIMP) was originally characterized as a protein

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($M_r$ 28 000) from rabbit bone culture medium (Cawston *et al.*, 1981), and is found in most tissues and cells in culture (Reynolds *et al.*, 1981). It can form a tight complex with the active forms of collagenase or stromelysin and may represent the final 'fail-safe' mechanism to control the extracellular activity of these enzymes (Reynolds, 1985; Murphy & Reynolds, 1985). Fibroblasts derived from both normal skin and hypertrophic scar tissue have been shown to synthesize and secrete TIMP into culture medium (Hembry, Murphy & Reynolds, 1985). It is therefore of interest to determine whether TIMP is present within hypertrophic scar tissues, or is synthesized by fibroblasts only as a response to *in vitro* culture conditions.

The occlusion of the microvasculature of hypertrophic scars contributes to a measurable hypoxia (Sloan *et al.*, 1978). In ischaemia or hypoxia of the heart, there is evidence to suggest that the lysosomal enzyme cathepsin D is an important agent in promoting cardiac damage leading to irreversible infarct and that it is released, with other lysosomal enzymes, during hypoxia (Decker *et al.*, 1977; Sasai *et al.*, 1982). Cathepsins D and B have also been demonstrated in extracellular sites in the chronic inflammatory tissue of the rheumatoid synovium (Poole *et al.*, 1976; Mort, Recklies & Poole, 1984). It is possible that the chronic hypoxia and connective tissue matrix remodelling that occurs in hypertrophic scars may give rise to extracellular cathepsin D and other lysosomal enzymes.

Using specific antisera to collagenase, TIMP and cathepsin D and indirect immunofluorescence, we have examined normal, hypertrophic and mature dermal scars to determine precisely the cellular localization of these antigens, and their distribution within the different scar types.

## METHODS

### *Chemicals*

Dulbecco's modified Eagle's medium (DMEM) was obtained from Whittaker M.A. Bioproducts (Walkersville, MD, U.S.A.) and foetal bovine serum (FBS) from Hyclone Laboratories (Logan, UT, U.S.A.). All sera were heated at 56°C for 30 min prior to use to inactivate complement. All other reagents were reagent grade.

### *Antibodies*

The sheep antibody to human TIMP, S-anti-TIMP (Hembry *et al.*, 1985) and the sheep antibody to collagenase, S-anti-CL (Hembry *et al.*, 1986) have been characterized previously. The sheep antibody to human lysosomal cathepsin D, S-anti-CD, has been characterized and used in a previous immunohistochemical study (Poole *et al.*, 1976). Pooled normal sheep serum (NSS) was used as a control. The second antibody for the indirect immunofluorescence, a monovalent Fab′ preparation, labelled with fluorescein isothiocyanate, of an antibody raised in a pig to sheep Fab′ fragments (pig-FITC), has been described previously (Hembry *et al.*, 1985).

### *Scar tissue*

Tissues were removed during reconstructive surgery after third degree burn injuries in eight patients, ranging in age from 3 to 17 years, at the Shriners Burns Institute, Boston. The scar tissues were classified as hypertrophic ($n = 4$), mature ($n = 2$) and normal scars ($n = 2$), and were taken at least 6 months after the original injury. All tissue specimens were processed as quickly as possible. Excised tissues were treated in one of the following ways: fixed in 10% formalin and processed for conventional paraffin wax histology; snap frozen in 7% gelatin in liquid nitrogen for 90 s; or incubated in tissue culture medium: small pieces of tissue were placed on metal grids in 3 cm culture dishes and incubated at 37°C for 24 h in DMEM with either 5% S-anti-TIMP, 5% S-anti-CL, 5% S-anti-CD or 5% NSS, to capture any secreted antigen. The culture medium was replaced with fresh DMEM containing 10% FBS, and the incubations continued for 24 h to remove unreacted sheep proteins. The tissue was then snap-frozen in 7% gelatin in liquid nitrogen for 90 s.

### *Immunohistochemical methods*

The method for indirect immunofluorescence on tissue sections has been described previously (Hembry *et al.*, 1986). Briefly, frozen sections 5 μm thick were cut, fixed in 4% paraformaldehyde for 30 min and washed repeatedly in phosphate buffered saline (PBS). Sections from tissue frozen directly after excision (not cultured tissue) were incubated for 30 min at room temperature with either S-anti-TIMP, S-anti-CL, S-anti-CD, or NSS sera diluted 1:20 with PBS. After washing repeatedly in PBS, the sections were incubated further in the second antibody, pig-FITC, for 30 min, then washed in PBS and mounted in PBS: glycerol containing *p*-phenylenediamine. Some sections were counterstained with eriochrome black (Difco, 1% v/v, 2 min) which produces a red fluorescent cytoplasm. Frozen sections from tissue previously cultured in antibody were fixed and incubated in the second antibody, pig-FITC, alone, and then counterstained and mounted as described. Sections were viewed on an inverted Zeiss IM 35 microscope fitted with epifluorescence and standard FITC filters. The slides were photographed on Kodak Ektachrome film ASA 400, uprated during processing to 1600 ASA. After the fluorescence studies the coverslips were removed, the sections stained with haematoxylin and eosin and examined with bright field optics. Photographs of these were taken on Ilford Pan F film rated at 32 ASA.

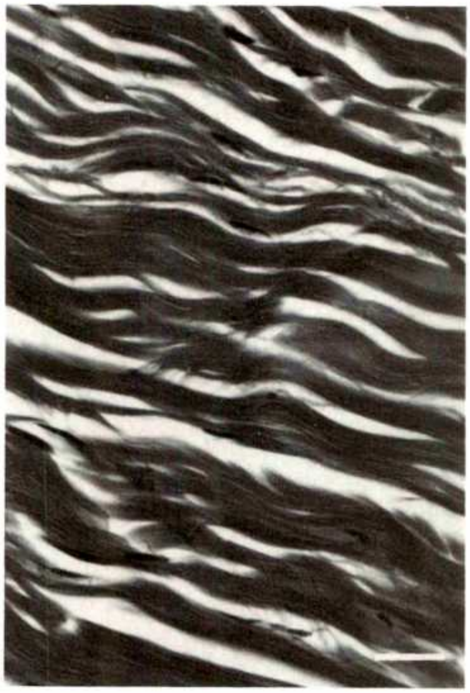

FIGURE 1. Paraffin wax section of inactive area of scar stained with haematoxylin and eosin. Dermal fibroblasts are sparse and spindle-shaped, with darkly staining nuclei. The collagen is thick and darkly stained. Bar = 20 μm.

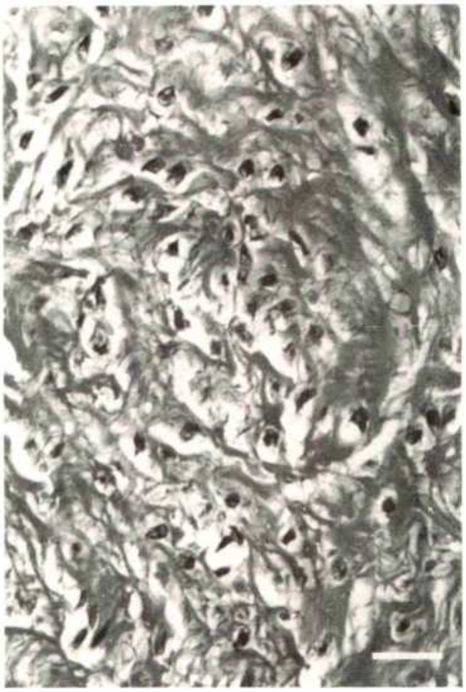

FIGURE 2. Paraffin wax section of area of scar undergoing active resorption, stained with haematoxylin and eosin. The many dermal fibroblasts present are pale and round. The collagen is fragmented, giving a lacy appearance. Bar = 20 $\mu$m.

## RESULTS

### *Collagenase localization*

Samples of normal, mature and hypertrophic scars contained nodules with inactive tissue (Fig. 1) and areas of active resorption (Fig. 2), sometimes in close proximity. Frozen tissues from all eight patients, sectioned, fixed and stained directly with S-anti-CL followed by pig-FITC, gave no immunofluorescent staining either on matrix components (collagen) or as immunoprecipitates. However, when these tissues were cultured with S-anti-CL for 24 h, sectioned, fixed and stained with pig-FITC, immunofluorescent staining was observed. Areas of scars judged to be inactive from their histological appearance (e.g. Fig. 1) contained negligible fluorescence (Fig. 3, top), but fine fluorescent immunoprecipitates were sometimes seen immediately around some dermal fibroblasts (Fig. 3, arrows), indicating that they were secreting low levels of collagenase. In contrast, areas of active resorption contained large amounts of immunoprecipitation throughout the area (Fig. 4). Fluorescent immunoprecipitates were also seen around some, but not all, dermal capillaries, including capillaries in otherwise negative areas (Fig. 5). The epidermis was always negative, and there was no evidence of increased collagenase immunofluorescence in the papillary dermis. All tissues cultured for 24 h in NSS before sectioning and staining with pig-FITC produced no immunofluorescent staining, showing that the 24 h incubation in DMEM with 10% FBS was sufficient to remove all unbound sheep serum proteins.

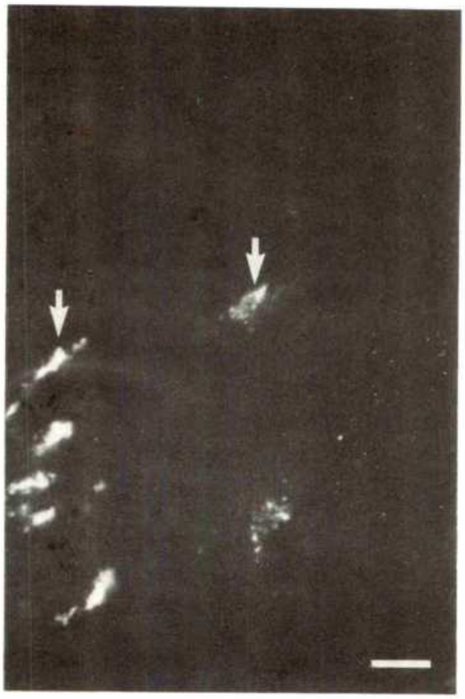

FIGURE 3. Collagenase immunofluorescence around dermal fibroblasts. Scar tissue was cultured in S-anti-CL for 24 h, and then in DMEM with 10% FBS for an additional 24 h. It was then frozen, sectioned and stained with pig-FITC. The area is generally negative, but dermal fibroblasts actively secreting collagenase fluoresce brightly (arrows). Bar = 20 $\mu$m.

### *TIMP localization*

Tissue from a mature scar sectioned, fixed and stained with S-anti-TIMP followed by pig-FITC, showed bright fluorescence of blood vessel endothelial cells (Fig. 6a). The contents of the blood vessel lumen also fluoresced (Fig. 6a, arrow). The same vessel stained with haematoxylin and eosin showed the endothelial cells to be few in number and near normal in appearance (Fig. 6b). Sections from all tissues stained with NSS followed by pig-FITC produced no immunofluorescence, showing that normal sheep serum proteins do not bind non-specifically to endothelial cells, vessel contents or connective tissue. When tissue from three of the four hypertrophic scars was stained as in Fig. 6a, however, no immunofluorescent staining of the blood vessel endothelial cells was seen in any of the sections. Histological sections from these three scars revealed that some of the vessels were occluded or partially occluded, and that the endothelial cells were numerous and enlarged, with crenated nuclei (Fig. 7). In other scar tissues, including one hypertrophic scar, TIMP immunofluorescence was present in the endothelial cells, but it was weaker and confined to the lumenal surface. The vessel contents also stained, suggesting patency of these vessels. The endothelial cells of these vessels, when stained with haematoxylin and eosin, had a near normal appearance.

When the scar tissues were cultured with S-anti-TIMP for 24 h, then sectioned and stained with pig-FITC, inactive areas of dermis contained negligible immunoprecipitation, although

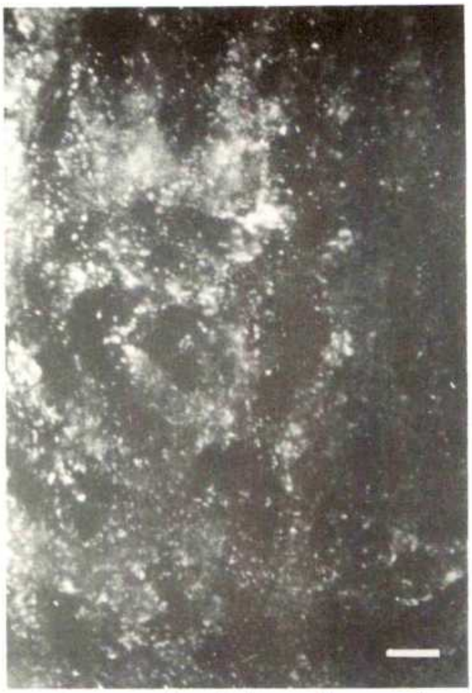

FIGURE 4. Collagenase immunofluorescence in actively resorbing area of scar. The scar tissue was treated as in Figure 3. Fluorescent immunoprecipitates of collagenase are visible throughout the area. Bar = 20 μm.

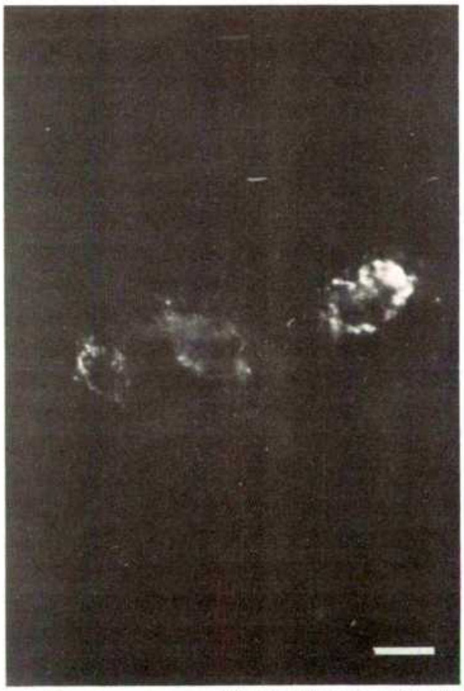

FIGURE 5. Collagenase immunofluorescence around dermal blood vessels. The scar tissue was treated as in Figure 3. Fluorescent immunoprecipitates of collagenase are concentrated around three dermal capillaries. Bar = 20 μm.

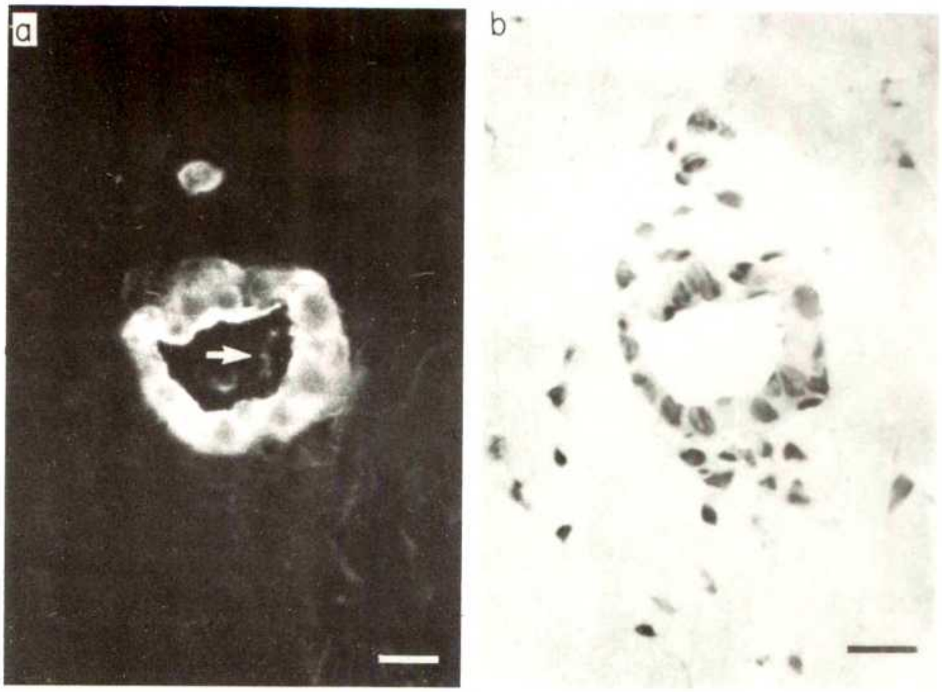

FIGURE 6. Blood vessel endothelial cells of mature scar. (a) TIMP immunofluorescence. Mature scar tissue was frozen, sectioned, fixed and stained with S-anti-TIMP followed by pig-FITC. The endothelial cells of the blood vessel and the vessel contents fluoresce brightly (arrowed). Bar = 20 μm. (b) The same vessel as in (a), but the frozen section was stained with haematoxylin and eosin. Bar = 20 μm.

the occasional cell was visible surrounded by immunoprecipitates, resulting in a picture similar to that shown for collagenase in Figure 3. However, in areas of active resorption, large amounts of immunoprecipitation were visible, particularly around blood vessels (Fig. 8). The endothelial cells of the vessels shown in Figure 8 did not fluoresce; histological staining of these vessels showed the lumina to be completely occluded by endothelial cells. The epithelium was always negative and there was no evidence of increased amounts of TIMP secretion in the papillary dermis. Therefore, as was found with collagenase, TIMP immunoprecipitation was found in all scar types in areas of apparent resorption and collagen breakdown; the inactive areas were predominantly negative.

### *Cathepsin D localization*

Sections from tissue frozen directly after surgery and stained with S-anti-CD followed by pig-FITC showed no immunofluorescence. Tissue cultured for 24 h in S-anti-CD showed occasional immunoprecipitates on the surface of the explant, and this was attributed to cells damaged during explanting. No immunoprecipitates were seen in the centre of the explants or around blood vessels. This demonstrates that cathepsin D is not present extracellularly in these tissues and, therefore, suggests that it does not play a significant role in the turnover of

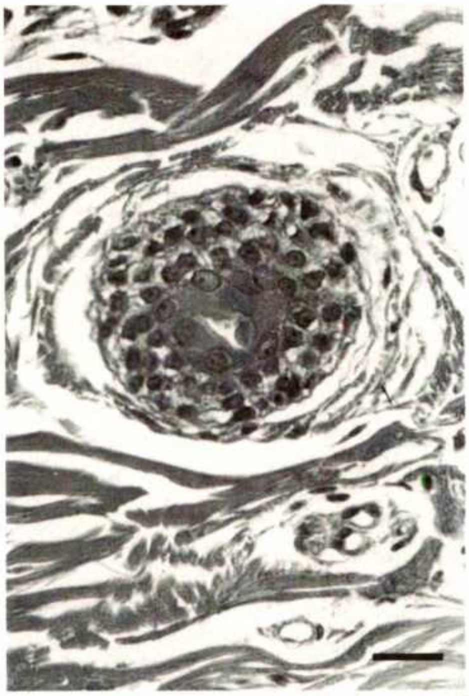

FIGURE 7. Paraffin wax section of a hypertrophic scar stained with haematoxylin and eosin. Note occlusion of the central blood vessel and numerous enlarged endothelial cells. Sections from this scar showed no TIMP immunofluorescence of the endothelial cells. Bar = 20 μm.

extracellular matrix in these scars. The sections were not permeabilized to allow antibody immunoglobulin to penetrate the cells, so that lysosomal cathepsin D was not seen.

## DISCUSSION

We have demonstrated, by indirect immunofluorescence, that collagenase and its naturally occurring inhibitor, TIMP, are present in human dermal scars. Little or no collagenase and TIMP were found in inactive areas of all scar types, whereas actively resorbing areas of all scars contained both.

Collagenase has been immunolocalized in human skin previously (Reddick, Bauer & Eisen, 1974; Woolley & Grafton, 1980) and was found, in apparent abundance, on dermal 'stromal elements'. It is now known, however, from biochemical studies, that human skin collagenase is secreted by cells in an inactive pro-enzyme form (Stricklin *et al.*, 1977), which does not bind to collagen fibrils (Stricklin *et al.*, 1978; Welgus *et al.*, 1985). Recent immunolocalization studies, using the same antiserum to collagenase as used in the present study, have confirmed that only active collagenase binds to, and is immunolocalizable on, collagen fibrils (Hembry *et al.*, 1986). This antiserum to collagenase does not distinguish between the active form and the pro-enzyme, or collagenase-TIMP complexes, but the pro-enzyme (and possibly collagenase-TIMP complexes) may be localized extracellularly as immunoprecipitates, as demonstrated previously

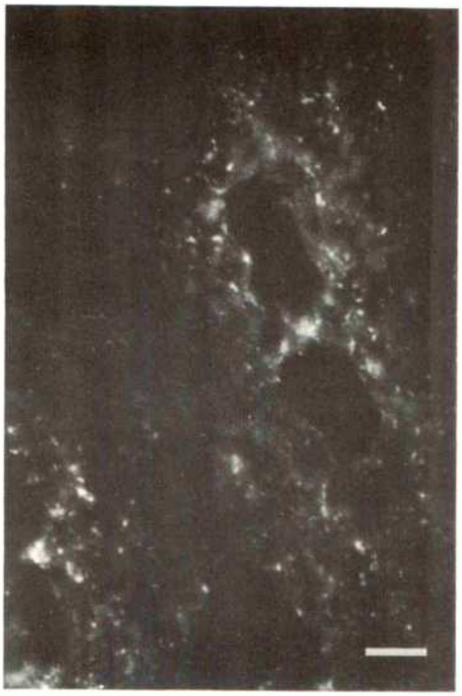

FIGURE 8. TIMP immunoprecipitation around the dermal blood vessels of hypertrophic scar. Hypertrophic scar (same scar as in Fig. 7) was cultured for 24 h with S-anti-TIMP followed by 24 h in DMEM and 10% FBS. It was then frozen, sectioned and stained with pig-FITC. Fluorescent immunoprecipitates are visible around the vessels, but not over the endothelial cells, which occlude completely the vessel lumen. Bar = 20 $\mu$m.

(Hembry *et al.*, 1986). The finding that areas of dermal scars, judged to be inactive by their histological appearance, contained no collagenase is in agreement with our observations on the non-gravid rabbit uterus (Hembry *et al.*, 1986). On the other hand, areas of the scars which appeared histologically to be actively remodelling, contained considerable collagenase immunoprecipitation, identical to that observed in the post-partum rabbit uterus which is known to undergo rapid resorption. Positive and negative areas of staining were often in close proximity, suggesting that the control of collagenase secretion is a very local event. No active collagenase bound to collagen was seen, which was in agreement with our observations on the post-partum rabbit uterus.

No collagenase secretion was observed in the epidermis of any of the scars examined. This agrees with the data of Eisen (1969), who showed that normal human epidermis gave minimal lysis when cultured on collagen gel substrates. There was also no evidence of increased collagenase immunoprecipitation in dermal tissue immediately underlying the epidermis. Johnson-Wint and Gross (1984) showed that epithelial cells in culture can stimulate dermal cells to synthesize more collagenase, and Ehrlich and Buttle (1984) showed that the epidermis promoted collagenase synthesis in long-term cultures of hypertrophic scars. Goslen, Eisen and Bauer (1985) have isolated a cytokine ($M_r$ 19 000) from basal cell carcinoma conditioned medium which stimulated collagenase production by skin fibroblasts in culture. The production of these factors by epidermal cells in culture suggests a potential mechanism for the regulation of collagenase synthesis, which may be tightly controlled *in vivo*.

This study is the first to demonstrate TIMP in tissue sections. TIMP secretion was observed, along with collagenase, in areas of scars which were undergoing active remodelling. This is consistent with the view that TIMP is produced by cells in tissues to control the extracellular activity of the metalloproteinases (Murphy & Reynolds, 1985). The major cell type in the hypertrophic scar is the fibroblast (Blackburn & Cosman, 1966; Linares *et al.*, 1972) and, from our observations, it is these cells that secrete collagenase and TIMP. The question arises whether the same cell is secreting both at the same time. Our studies with human skin fibroblasts in culture show that under some conditions the metalloproteinases and TIMP are secreted simultaneously (unpublished observation), but further studies, using dual immunolocalization techniques, are necessary to determine whether this occurs *in vivo*.

We have also demonstrated that the endothelial cells of dermal blood vessels contain TIMP. Biochemical data have shown that human endothelial cells in culture produce TIMP (Dwyer, Herron & Banda, 1985). The blood vessel endothelial cells of normal human skin, placenta and rheumatoid synovium also immunofluoresce strongly when stained as described in these experiments (data not shown), suggesting that TIMP is a normal constituent of these cells. The observation that the endothelial cells of the occluded vessels of three of the hypertrophic scars did not stain is interesting. The small number of scars studied here gives no indication of whether the absence of TIMP is a cause or a result of vessel occlusion, and further biochemical studies on the factors affecting endothelial cell TIMP secretion are necessary before we can say whether this finding has any significance.

TIMP was also observed in the lumen of many blood vessels. TIMP-like inhibitors have been isolated from serum (Bunning *et al.*, 1984) and a collagenase inhibitor immunologically identical to TIMP has also been isolated and characterized from human platelets (Cooper *et al.*, 1985). TIMP in serum constitutes only a minor proportion of the anti-collagenase activity of serum, the major being $\alpha_2$-macroglobulin. Little is known about the fate of collagenase-TIMP complexes once they have been formed in the tissue; they may be phagocytosed like protease nexin-thrombin complexes (Murphy & Reynolds, 1985) or they may be transported to the blood vessels and removed in the serum. We observed considerable TIMP and collagenase immunoprecipitation around some scar blood vessels which might support the latter view, but which may also be a result of the general leakage of plasma proteins from the microvasculature in these tissues (Kischer *et al.*, 1983).

## ACKNOWLEDGMENTS

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# An open study of vitamin $D_3$ treatment in psoriasis vulgaris

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## SUMMARY

Active forms of vitamin $D_3$, 1α-hydroxyvitamin $D_3$ and 1α,25-dihydroxyvitamin $D_3$, were administered in an open-design study to 40 patients with psoriasis vulgaris in three ways: (1) to 17 patients 1α-hydroxyvitamin $D_3$ was given orally at a dose of 1·0 μg/day for 6 months, (2) to four patients 1α,25-dihydroxyvitamin $D_3$ was given orally at a dose of 0·5 μg/day for 6 months, and (3) 19 patients were given 1α,25-dihydroxyvitamin $D_3$ applied topically at a concentration of 0·5 μg/g of base for 8 weeks. Improvement was observed at the end of the individual study periods in 13 (76%) patients in Group 1 with a mean period of treatment (± SD) of 2·7 ± 0·6 months, in one patient in Group 2 at 3 months after the start of treatment, and in 16 (84%) patients in Group 3 when the chemical was applied for 3·3 ± 1·2 weeks. No side-effects were observed in any of these trials. These data suggest that psoriasis may respond to active metabolites of vitamin $D_3$ and that abnormalities in vitamin D metabolism or in responsiveness of the skin cells to active metabolites of vitamin D may be involved in the pathogenesis of this skin disease.

The cause of the increase in epidermal cellular turnover in psoriasis is unkown, but is believed to be due to loss of the normal mechanism for regulation of cell division (Weinstein & Frost, 1968; Chopra & Flaxman, 1974). It is recognized that the skin plays an important role in vitamin D metabolism. 7-Dehydrocholesterol, provitamin $D_3$, is converted in the skin to previtamin $D_3$ by a photochemical reaction under irradiation with ultraviolet light, and the latter then undergoes thermal isomerization to vitamin $D_3$ (Holick *et al.*, 1977; Esvelt, Shnoes & DeLuca, 1978; Holick *et al.*, 1979). Vitamin $D_3$ is metabolized further by sequential hydroxylation: first in the liver at the 25-position to 25-hydroxyvitamin $D_3$, and then by hydroxylation in the kidney to 24R, 25-dihydroxyvitamin $D_3$ and 1α,25-dihydroxyvitamin $D_3$ (1,25-$(OH)_2D_3$). The latter is known to be the hormonally active form in enhancing intestinal calcium (Ca) transport and bone mineral mobilization (Kodicek, 1974; Norman & Henry, 1974; DeLuca & Schnoes, 1976).

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There is also recent evidence that, besides the intestine and bones, the skin itself is a target organ of 1,25-$(OH)_2D_3$. Tritiated 1,25-$(OH)_2D_3$ was found to accumulate in the nuclei of skin cells (Stumpf *et al.*, 1979), and epidermal keratinocytes and dermal fibroblasts were found to have a specific receptor for 1,25-$(OH)_2D_3$ (Colston, Hirt & Feldman, 1980; Feldman *et al.*, 1980; Simpson & DeLuca, 1980, Clemens *et al.*, 1981; Eil & Marx, 1981; Clemens *et al.*, 1983). The significance of accumulation of 1,25-$(OH)_2D_3$ and the presence of the receptor in the skin were unknown until recently, when Hosomi *et al.* (1983) showed that a physiological concentration of 1,25-$(OH)_2D_3$ regulated terminal differentiation of epidermal keratinocytes: 1,25-$(OH)_2D_3$ strongly stimulated differentiation of basal cells into squamous and enucleated cells *in vitro*.

We observed a patient with senile osteoporosis complicated by psoriasis vulgaris (Morimoto & Kumahara, 1985), in whom the psoriasis was cured by oral administration of 1α-hydroxyvitamin $D_3$ (1α-$OHD_3$), a synthetic precursor of 1,25-$(OH)_2D_3$ (Holick *et al.*, 1973; Barton *et al.*, 1973; Holick *et al.*, 1975; Fukushima *et al.*, 1975), prescribed initially for osteoporosis. This observation prompted us to confirm the effect by an open-design study. This paper reports the results of this study in which active forms of vitamin $D_3$, namely 1α-$OHD_3$ and 1,25-$(OH)_2D_3$, were given orally and 1,25-$(OH)_2D_3$ was applied topically to patients with psoriasis vulgaris.

## METHODS

Forty patients with psoriasis vulgaris were studied. These patients consisted of 34 males and six females with a mean age of 44 years (range 15–83). They had been suffering from psoriasis vulgaris for a mean of 8·6 years (range 0·8–35 years). None of them had any serious disease besides psoriasis vulgaris. The diagnosis of psoriasis was made on the clinical appearance of the skin lesions characterized by erythematous plaques of various sizes with silver-white scales. Previous treatment had included topical corticosteroid preparations and ultraviolet irradiation, without a satisfactory result. Before starting administration of active forms of vitamin $D_3$, all other medications for psoriasis vulgaris were stopped. No medication that might alter Ca metabolism was given to any of these patients. Each subject was informed of the experiment and gave written consent. These patients, grouped randomly, were treated with active forms of vitamin $D_3$ in the following three ways: (1) 17 patients were given 1α-$OHD_3$ (Alfarol®, Chugai Pharmaceutical Co., Tokyo, Japan) orally at a dose of 1·0 μg/day for 6 months, (2) four patients were given 1,25-$(OH)_2D_3$ (Nippon Roche Co. Ltd, Tokyo, Japan) orally at a dose of 0·5 μg/day for 6 months, and (3) 19 patients were given 1,25-$(OH)_2D_3$ (Chugai Pharmaceutical Co.) topically at a concentration of 0·5 μg/g in a base (petrolatum) once a day, followed by occlusive dressing with polyethylene film for 12 h, for 8 weeks. As a control, the base only was applied to the contralateral skin lesions in the same manner.

The patients treated orally were examined once every 2 weeks, and those treated topically were examined once a week to determine the effects of treatments. The effects of treatments were evaluated by comparing clinical photographs taken at every examination, and were classified as complete remission (+4), marked improvement (+3), moderate improvement (+2), slight improvement (+1), no change (0) and deterioration (−1). The patients who showed improvement of more than +2 were classified as responders, and those who showed improvement of less than +1 as non-responders. Hepatic and renal function, as well as the serum Ca and inorganic phosphate (Pi), levels were monitored at the time of examination. Blood sampling for assays was done before and during the study at 8.00–9.00 a.m. after the patients had fasted overnight. Sera obtained by centrifugation were frozen and kept at −20°C until assayed.

### *Analytical procedures*

The serum level of 25-hydroxyvitamin D (25-OHD) was determined by competitive protein binding assay, as described by Shimotsuji and Seino (1980). The serum level of 1α,25-dihydroxyvitamin D (1,25-$(OH)_2D$) was measured by radioreceptor assay by the method of Eisman *et al.* (1976) and Seino *et al.* (1980), with some modifications (Lee *et al.*, 1982). The overall recoveries of 25-OHD and 1,25-$(OH)_2D$ were 72·0 ± 10·3% and 65·0 ± 9·2% respectively, and the coefficients of variation within and between assays were 7·8% and 15·0% respectively in the 25-OHD assay, and 12·1% and 18·0% in the 1,25-$(OH)_2D$ assay. Serum parathyroid hormone (PTH) was measured with a commercially available radioimmunoassay kit (Eiken Immunochemical Lab., Tokyo, Japan) using antiserum directed towards the carboxy-terminal portion of the peptide (Morimoto *et al.*, 1980). The sensitivity of the assay was 100 pg bovine PTH eq/ml and the within- and between-assay coefficients of variation were 5% and 17% respectively. Plasma human calcitonin (hCT) was measured by radioimmunoassay as described previously (Morimoto *et al.*, 1980) with antiserum directed towards the mid-portion of the peptide; the sensitivity was 49 pg/ml, and the within- and between-assay coefficients of variation were 8% and 22%, respectively. The serum levels of Ca and Pi were measured with a multichannel Technicon autoanalyser by the methods of Gitelman (1976) and Fiske and Subbarow (1925). The serum levels of these parameters in the psoriatic patients were compared to those of 24 age-matched normal subjects (19 men and 5 women with a mean age of 45 years; range 28–60 years). All samples from patients were examined in the same assay to reduce between-assay variation. Values were expressed as means ± SD. Student's two-tailed *t*-test was used for statistical analyses.

## RESULTS

### *Effects of active metabolites of vitamin $D_3$ on psoriasis*

Table 1 summarizes the effectiveness of treatment with 1α-$OHD_3$ and 1,25-$(OH)_2D_3$ on psoriatic skin lesions, and the mean duration of administration of the individual active forms of vitamin $D_3$ to patients whose skin lesions showed clearance or marked improvement ($\geqslant +2$). Improvement of more than +2 at the end of the individual observation periods was observed in 13 of 17 patients (76%) given 1α-$OHD_3$ orally (1·0 μg/day), in one of four patients (25%) given 1,25-$(OH)_2D_3$ orally (0·5 μg/day), and in 16 of 19 patients (84%) treated with 1,25-$(OH)_2D_3$ topically (0·5 μg/g in a base). The appearance of the skin of one patient before and during oral treatment with 1α-$OHD_3$ is shown in Figure 1. Slight improvement or less, of skin lesions after 6 months treatment was observed in the other seven patients given 1α-$OHD_3$ or 1,25-$(OH)_2D_3$ orally, and in three patients after topical application of 1,25-$(OH)_2D_3$ for 8 weeks. In all patients with topical application, no improvement was seen in the contralateral skin lesions treated with vehicle only.

### *Effects of active metabolites of vitamin $D_3$ on serum levels of Ca, Pi, PTH, CT, 25-OHD and 1,25-$(OH)_2D$*

The effects of these treatments with active forms of vitamin $D_3$ on the serum levels of Ca, Pi, PTH, CT, 25-OHD and 1,25-$(OH)_2D$ are summarized in Table 2. The mean basal values of all these parameters were similar in the psoriatic patients and age-matched normal subjects. The mean serum levels of Ca, Pi, 1,25-$(OH)_2D$ in the group given 1α-$OHD_3$ orally, and that of Ca in the group given 1,25-$(OH)_2D_3$ orally, were elevated significantly 3 months after the start of treatment, which was near the mean time of treatment for an improvement of more than +2 in

TABLE 1. Efficacy of treatment of psoriasis vulgaris with active metabolites of vitamin $D_3$

| Treatment | No. of patients (M/F) | Age (years) (mean ± SD) (range) | Degree of improvement | | | | | | No. of patients | | Effective therapeutic period** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | −1 | 0 | +1 | +2 | +3 | +4 | Treatment effective* | Treatment ineffective | |
| Oral<br>1α-$OHD_3$<br>1·0 μg/day | 17 14/3 | 47 ± 12 (23–63) | 1 | 2 | 1 | 4 | 4 | 5 | 13 | 4 | 2·7 ± 0·6 months |
| Oral<br>1,25-$(OH)_2D_3$<br>0·5 μg/day | 4 4/0 | 38 ± 19 (18–55) | 0 | 2 | 1 | 1 | 0 | 0 | 1 | 3 | 3 months |
| Topical<br>1,25-$(OH)_2D_3$<br>0·5 μg/g | 19 16/3 | 48 ± 18 (18–83) | 0 | 0 | 3 | 7 | 6 | 3 | 16 | 3 | 3·3 ± 1·2 weeks |

* Treatment effective: ⩾ +2.

** Mean period of administration ± SD until improvement of more than +2 was observed.

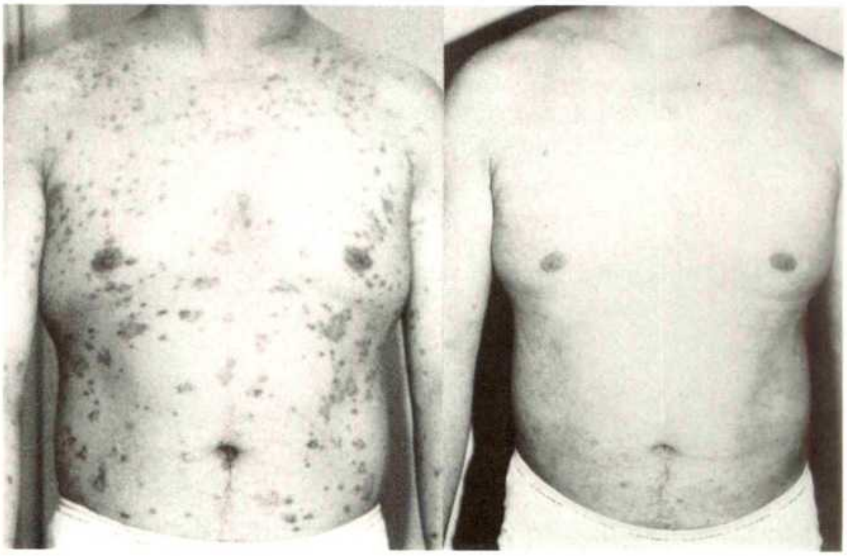

FIGURE 1. A 37-year-old man who had been suffering from psoriasis vulgaris for 10 years. Left: before treatment, right: 4 months after the start of oral administration of $1\alpha$-$OHD_3$ at a dose of 1·0 μg/day.

TABLE 2. Serum levels of Ca, Pi, PTH, CT, 25-OHD and 1,25-$(OH)_2D$, before and during administration of active metabolites of vitamin $D_3$. Values are means ± SD

| Treatment | No. of subjects | | Ca (mg/dl) | Pi (mg/dl) | PTH (pgeq/ml) | hCT (pg/ml) | 25-OHD (ng/ml) | 1,25-$(OH)_2D$ (pg/ml) |
|---|---|---|---|---|---|---|---|---|
| Oral | 17 | b* | 9·2 ± 0·6 | 3·8 ± 0·8 | 260 ± 50 | 53 ± 17 | 23 ± 12 | 41 ± 19 |
| $1\alpha$-$OHD_3$ | | d | 9·6 ± 0·6 | 4·2 ± 0·4 | 250 ± 50 | 51 ± 11 | 27 ± 18 | 62 ± 32 |
| (1·0 μg/day) | | | $P < 0·001$† | $P < 0·005$ | NS | NS | NS | $P < 0·05$ |
| Oral | 4 | b | 9·4 ± 0·2 | 3·8 ± 0·8 | 270 ± 60 | 59 ± 13 | 17 ± 5 | 40 ± 11 |
| 1,25-$(OH)_2D_3$ | | d | 9·8 ± 0·2 | 3·7 ± 0·8 | 200 ± 100 | 58 ± 37 | 15 ± 8 | 47 ± 12 |
| (0·5 μg/day) | | | $P < 0·05$ | NS | NS | NS | NS | NS |
| Topical | 19 | b | 9·3 ± 0·2 | 4·1 ± 0·8 | 280 ± 100 | 54 ± 12 | 20 ± 10 | 34 ± 11 |
| 1,25-$(OH)_2D_3$ | | d | 9·2 ± 0·4 | 4·0 ± 0·6 | 260 ± 50 | 60 ± 20 | 20 ± 9 | 35 ± 12 |
| (0·5 μg/g) | | | NS | NS | NS | NS | NS | NS |
| Psoriatic group | 40 | b | 9·2 ± 0·6 | 3·9 ± 0·9 | 270 ± 80 | 54 ± 17 | 21 ± 11 | 38 ± 15 |
| (mean) | | | NS‡ | NS | NS | NS | NS | NS |
| Normal subjects | 24 | | 9·3 ± 0·4 | 3·7 ± 0·4 | 270 ± 60 | 55 ± 17 | 21 ± 15 | 41 ± 14 |
| Normal range | | | 8·4–10·2 | 3·0–4·5 | 180–460 | < 150 | 7–35 | 20–60 |

* :b, before and d, during treatment (3 months on oral administration and 3 weeks on topical application).

† :Significance of difference between values before and during treatment using a paired *t*-test.

‡ :Significance of difference between basal levels in psoriatic patients and levels in normal subjects using an unpaired *t*-test.

NS: not significant.

these groups. Topical application of 1,25-$(OH)_2D_3$ for 3 weeks did not affect any of these parameters, although this treatment resulted in an average improvement of more than +2.

### *Absence of side-effects of 1α-$OHD_3$ and 1,25-$(OH)_2D_3$*

None of the patients in the three groups suffered from any topical or systemic complications or symptoms during these observation periods. Blood and urine analyses showed values within normal limits at all times. Hepatic and renal function, evaluated by measuring the serum levels of glutamic oxaloacetic transferase, glutamic pyruvic transferase, urea nitrogen and creatinine, were within normal ranges and did not change significantly during the observation periods.

## DISCUSSION

None of the currently available treatments for psoriasis are satisfactory, as they are either time-consuming, expensive, only temporarily effective, or even potentially carcinogenic. This study demonstrated the possible efficacy of biologically active metabolites of vitamin $D_3$ for treatment of patients with psoriasis vulgaris, and indicated that oral administration of 1α-$OHD_3$ and topical application of 1,25-$(OH)_2D_3$ are useful treatments. No cutaneous or systemic side-effects were seen, such as those observed on retinoid treatment (Tsambaos & Orfanos, 1981). One patient treated orally with 1,25-$(OH)_2D_3$ showed moderate improvement after treatment for 3 months, but only four patients received this treatment and so its efficacy requires further study. The dose of 1,25-$(OH)_2D_3$ used was half that of 1α-$OHD_3$, since 1,25-$(OH)_2D_3$ is nearly twice as effective as 1α-$OHD_3$ on a molar basis when applied *in vivo* (Holick *et al.*, 1975). However, oral 1α-$OHD_3$ at a dose of 1·0 μg/day may be more effective than oral 1,25-$(OH)_2D_3$ at a dose of 0·5 μg/day, because the latter may be trapped in the intestine and so not reach the skin at an effective concentration, while 1α-$OHD_3$ is metabolized to 1,25-$(OH)_2D_3$ in the liver (Fukushima *et al.*, 1975).

The mechanism of the effects of 1,25-$(OH)_2D_3$ and 1α-$OHD_3$ is still unknown. A relationship between psoriasis and Ca metabolism has been suggested in several publications. Hypoparathyroidism may be cause of the onset or aggravation of psoriasis in patients with surgical hypoparathyroidism (Vickers & Sneddon, 1963; Montgomery, 1964), primary hypoparathyroidism (Risum, 1973) and pseudohypoparathyroidism (Laymon & Zelickson, 1959). Chronic and acute hypocalcemia were also shown repeatedly in patients with pustular psoriasis (Copeman & Bold, 1965; Baker & Ryan, 1968; Stewart, Battaglini-Sabetta & Millstone, 1984), and the pustular psoriasis subsided on restoration of the circulating Ca level to the normal range (Stewart, Battaglini-Sabetta & Millstone, 1984). The patients examined in this study did not have any history of endocrinological disorder, and their basal levels of serum Ca were within the normal range.

Although the serum Ca level increased significantly during treatment in the groups given 1α-$OHD_3$ and 1,25-$(OH)_2D_3$ orally, the increases were slight and the levels remained within the normal range. Moreover, no increase in serum Ca level was observed in the group treated topically with 1,25-$(OH)_2D_3$ in which comparable improvement was observed. We also found that there were no significant differences in the mean basal levels of circulating Pi, PTH, CT, 25-OHD and 1,25-$(OH)_2D$, in psoriatic patients and normal subjects. This suggests that changes in the circulating levels of Pi, PTH, CT and vitamin D metabolites and of Ca, do not contribute to the improvement of psoriasis vulgaris by the active forms of vitamin $D_3$, at least in the patients studied in this work, although hypocalcemia caused by hypoparathyroidism is reported to cause or aggravate the skin lesions (Risum, 1973), and in these patients the circulating level of 1,25-$(OH)_2D_3$ may be abnormally low.

Since the most characteristic feature of psoriatic lesions is abnormally increased proliferation of epidermal keratinocytes, all the above evidence suggests strongly that the hormonally active form of vitamin $D_3$ directly affects the psoriatic skin lesions, suppressing proliferation and stimulating differentiation of the cells in the epidermis. Recent studies have also shown that 1,25-$(OH)_2D_3$, besides having an effect on Ca metabolism, suppresses proliferation and induces differentiation of certain cells that have a specific receptor for this hormone; 1,25-$(OH)_2D_3$ inhibits *in vitro* growth of proliferating cells such as malignant melanoma cells (Colston, Colston & Feldman, 1981), breast cancer cells (Iwaki, Iwaki & Freake, 1983), and even cultured dermal fibroblasts (Clemens *et al.*, 1983). Suda's group was the first to demonstrate induction of differentiation; they found that it induced differentiation of myeloid leukemia cells of mice (M-1 cells) (Abe *et al.*, 1981), and humans (HL-60 cells) (Miyaura *et al.*, 1981; Tanaka *et al.*, 1982), both of which have a specific receptor for 1,25-$(OH)_2D_3$. Administration of 1,25-$(OH)_2D_3$ or 1α-$OHD_3$ to mice inoculated with M-1 cells prolonged their survival time (Homma *et al.*, 1983). In addition, they showed that 1,25-$(OH)_2D_3$ at a concentration of 0·12 nM or more stimulated terminal differentiation of epidermal keratinocytes (Hosomi *et al.*, 1983); basal cells treated with 1,25-$(OH)_2D_3$ decreased in number and differentiated into squamous and enucleated cells. Recently, MacLaughlin *et al.* (1985) reported that cultured dermal fibroblasts from patients with psoriasis have partial resistance to the inhibitory effect of 1,25-$(OH)_2D_3$ on cell proliferation.

The present results indicate the possible effectiveness of oral 1α-$OHD_3$ 1,25-$(OH)_2D_3$ and topical 1,25-$(OH)_2D_3$ on psoriatic lesions. A double-blind study is needed now to elucidate the efficacy of hormonally active forms of vitamin D in treatment of patients with psoriasis vulgaris. The mechanism of regulation of terminal differentiation of psoriatic skin by 1,25-$(OH)_2D_3$ has also still to be examined.

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# Successful treatment of psoriasis with topical application of active vitamin $D_3$ analogue, 1α,24-dihydroxycholecalciferol

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## SUMMARY

We treated 11 psoriatic patients with topical 1α,24-dihydroxycholecalciferol, a new synthetic analogue of active vitamin $D_3$. In 10 of 15 tests the lesions cleared completely within 1–4 weeks, although some relapses occurred shortly after cessation of treatment. There were no side-effects. We suggest that 1α,24$(OH)_2D_3$ merits further investigation as a potentially useful topical therapy for psoriasis.

An effective topical therapeutic modality for psoriasis which has no side-effects, such as are found with corticosteroids, is still needed. Recently, Morimoto *et al.* (1985) found that in a patient with senile osteoporosis, associated psoriatic skin lesions disappeared unexpectedly during treatment with oral active vitamin $D_3$ (1α-OH-$D_3$). Subsequently, in 12 psoriatic patients they observed a beneficial effect with systemic or topical administration of 1α-OH-$D_3$ or 1,25$(OH)_2D_3$ . 1α-OH-$D_3$ is a prodrug that has to be metabolized in the liver to active 1α,25$(OH)_2D_3$; a new synthetic analogue 1α,24$(OH)_2D_3$ (1α,24-dihydroxycholecalciferol, Teijin Ltd, Tokyo, Japan) is as active as natural 1α,25$(OH)_2D_3$ in mineral metabolism, but less toxic than 1α-OH-$D_3$ (Kawasaki *et al.*, 1977). We present the results of a preliminary study in which we assessed the efficacy of topical 1α,24$(OH)_2D_3$ in psoriasis.

## METHODS

Eleven patients with psoriasis (eight males, three females; aged 15–84 years) participated in the study after giving informed consent. Most of the patients had had widespread skin lesions for 1–25 years that were relatively resistant to various forms of treatment, including PUVA and oral etretinate.

1α,24$(OH)_2D_3$ ointments containing 1, 2, or 4 μg/g of petrolatum were prepared by the Teijin Institute for Biomedical Research, Tokyo, Japan, and applied to lesions in localized areas.

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Responses were scored as: 0, no response; 1, moderate improvement; 2, marked improvement; and 3, complete clearing.

## RESULTS

Application of 4 μg/g $1\alpha,24(OH)_2D_3$ ointment under occlusion to psoriatic lesions on the right forearm of a 20-year-old female patient induced a rapid clearance within 14 days, whereas only slight improvement was found in control lesions on the left forearm treated with petrolatum also under occlusion.

Application of the 4 μg/g ointment twice daily also cleared lesions on the right ear of a 15-year-old girl within 7 days, whereas no apparent change occurred in the lesions on the left ear treated with petrolatum only.

Seven tests were then performed with $1\alpha,24(OH)_2D_3$ ointment containing between 1 and 4 μg/g under 12-h occlusion, applied to lesions located on the extremities in seven patients. As a control, 0·12% betamethasone 17-valerate ointment was applied in the same manner to the lesions symmetrically located on the contralateral limbs. Again, the application of $1\alpha,24(OH)_2D_3$ ointment under occlusion produced improvement in all the skin lesions within one month, irrespective of the concentration used. In five of seven patients, treated psoriatic lesions cleared almost completely. Improvement in lesion scores with active vitamin $D_3$ ointment and with 0·12% betamethasone 17-valerate ointment were almost the same (mean ± SD, 2·7 ± 0·4 and 2·5 ± 0·5, respectively) although it took longer for clearing of the lesions treated with the $1\alpha,24(OH)_2D_3$ ointment than those treated with the corticosteroid ointment (mean ± SD, 18 ± 5·5 days and 12 ± 2·8 days, respectively).

In a further eight tests $1\alpha,24(OH)_2D_3$ ointment was applied twice daily to a limited portion of the face or scalp in three patients and to the trunk or limbs in five patients. This also produced an excellent effect in five of the eight patients within one month. The skin lesions on the seborrhoeic areas, including the forehead, scalp and ear, cleared almost completely with 2μg/g or 4μg/g ointment. Time for clearing of the lesions ranged from 7 to 21 days, mean 18 days. In the remaining three patients with test sites on the forearm, buttocks and face, respectively, the clinical response was rather poor, even with the 4 μg/g ointment.

A 3 week follow-up showed a mild relapse in four of 11 patients who had shown an excellent response to topical $1\alpha,24(OH)_2D_3$.

## DISCUSSION

At present, the exact mechanism of action of $1\alpha,24(OH)_2D_3$ in psoriasis is not clear. Recently, the role of vitamin $D_3$ in cell proliferation and differentiation has caught the attention of many researchers (Kuroki, 1985). The skin is also known to have specific receptors for $1\alpha,25(OH)_2D_3$ (Feldman *et al.*, 1980); in the presence of active $D_3$, basal cells have been shown to undergo differentiation into squamous cells (Hosomi *et al.*, 1983). Rapid disappearance of scale and infiltration in psoriatic lesions, despite the persistence of slight erythema in our patients, suggests that $1\alpha,24(OH)_2D_3$ may induce prompt differentiation of highly proliferative psoriatic epidermal cells into squamous cells. Furthermore, its inhibitory action of specific immunological activities (Komoriya *et al.*, 1985) may have a beneficial effect on immunological processes taking place in psoriatic lesions. A preliminary study ruled out any measurable effect of vitamin $D_3$ on polymorphonuclear leukocyte function (unpublished data).

In the present study, no elevation of serum calcium or phosphorus levels was found after topical application of $1\alpha,24(OH)_2D_3$ for several weeks. Although we did not encounter any adverse effects during topical application to localized areas for up to 4 weeks, $1\alpha,24(OH)_2D_3$ should be used with caution until more data are available regarding its safety.

If it is confirmed that there are no serious side-effects with prolonged use, we think that topical $1\alpha,24(OH)_2D_3$ could be a potent and useful therapeutic modality in the treatment of psoriasis, particularly for lesions on the face and scalp where we have often observed severe side-effects with topical corticosteroids.

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## ADDENDUM

Since the present paper was accepted for publication, another report of the treatment of psoriasis with active vitamin $D_3$ has appeared (Morimoto *et al.*, 1986).

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# Circulating antibodies and antigenic cross-reactivity in *Hendersonula toruloidea* and *Scytalidium hyalinum* infections

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## SUMMARY

Twelve (34%) of thirty-five patients with an active infection on the palms or soles caused by *Hendersonula toruloidea* or *Scytalidium hyalinum* were found to have circulating antibodies to these organisms by counter immunoelectrophoresis or immunodiffusion, compared with 9% of uninfected controls. In every instance there was cross-reactivity between the positive patients' sera and the heterologous non-dermatophyte antigen.

Using crossed and intermediate gel immunoelectrophoresis it was found that cytoplasmic extracts of *H. toruloidea* and *S. hyalinum* showed 34 and 41 precipitin peaks respectively, most of which were shared by both organisms. No cross-reactions were observed between the non-dermatophyte cytoplasmic extracts and hyperimmune animal antisera raised to *Trichophyton rubrum*, *Trichophyton interdigitale*, *Aspergillus fumigatus* or *Candida albicans*. Exoantigens prepared from the two non-dermatophytes showed similar cross-reactivity between the two species. It appears that *H. toruloidea* and *S. hyalinum* are very similar in antigenic structure, a finding which lends support to the view that they may be closely related. They are also antigenically distinct from other pathogenic fungi which commonly infect skin, a fact which may be useful in their cultural identification as well as their recognition in tissue specimens.

Superficial infections caused by *Hendersonula toruloidea* (Gentles & Evans, 1970) and *Scytalidium hyalinum* (Campbell & Mulder, 1977) are virtually clinically indistinguishable from the chronic non-inflammatory 'dry' type of dermatophytosis of the palms and soles caused by *Trichophyton rubrum*. In some parts of the tropics these fungi may be very common causes of disease; a small survey of an unselected population on the island of Tobago, West Indies, found these organisms implicated in toe web or sole infections in at least 28% of asymptomatic subjects investigated (Allison, Hay & Campbell, 1984). A recent survey of *H. toruloidea* and *S. hyalinum* infections diagnosed in the U.K. has shown that they are restricted typically to palms, soles, toe webs and nails and are characterized by diffuse scaling and some erythema, but that overall clinical signs of inflammation are minimal (Hay & Moore, 1984). Despite this, 55% of those

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studied reported itching at the site of infection. Like 'dry' type dermatophytosis, these infections are chronic, an observation which has been noted by most investigators (e.g. Carruthers, Stein & Black, 1982). These findings suggest, therefore, that the host's response against these organisms is ineffective, or deficient.

*H. toruloidea* and *S. hyalinum* cause clinically identical infections and have a similar characteristic appearance on direct microscopy (Moore, 1978). Despite certain morphological differences in culture, and differences in their geographical distribution and ecology—unlike *H. toruloidea*, *S. hyalinum* has never been isolated from the environment—it is possible that the two organisms are closely related, if not varieties of the same species. To examine the nature and specificity of the immune response in these infections, the serological cross-reactivity between these organisms and certain other fungi was studied and the antibody responses in serum from infected patients were investigated.

## METHODS

### *Antigens*

*Cytoplasmic extracts*. Cytoplasmic extracts were prepared from isolates of *H. toruloidea*, *S. hyalinum*, *T. rubrum* and *T. interdigitale*. The same isolates of *H. toruloidea* (St. John's/MKM 7) and *S. hyalinum* (St. John's/MKM 1) were used throughout the study.

Fungi were grown in glucose/peptone broth at 28°C for 12 days, when the mycelial mat covered the surface of the broth. The mat was then harvested by filtration through cotton gauze, washed twice with sterile distilled water and ground for 2 min in an Atomix blender with cold volatile buffer (0·8% ammonium hydrogen carbonate). The resulting slurry was ground for 1 min with ballotini beads in a Dyno-Mill (KDL, Basel, Switzerland) with the grinding chamber surrounded by a cooling jacket containing circulant at 4°C and emptied from the chamber. The suspension was collected and the glass beads were washed several times with cold buffer. The washings and the supernatant fluid were retained and centrifuged for 1 h at 19 000 ***g*** and 4°C. The resulting supernatant was filtered through a 0·45 $\mu$m membrane (Millipore, Harrow, Middx, U.K.), dialysed at 4°C for 24 h against distilled water and freeze dried. Commercial cytoplasmic antigens of *Candida albicans* (CA, Mercia Brocades, Weybridge, Surrey, U.K.) and *Aspergillus fumigatus* (AF, Bencard, Brentford, Essex, U.K.) were used in the studies.

*Exoantigens*. As *H. toruloidea* is morphologically very variable in culture and isolates from infected patients may be differentiated into three colonial forms depending on their rate and pattern of growth (Moore, unpublished observations), a larger number of isolates was examined using exoantigens prepared after the methods of Standard and Kaufman (1976). Exoantigens are diffusable antigenic substances which can be eluted from growing cultures by aqueous solutions.

Exoantigens were prepared from the three morphological forms of *H. toruloidea* (nine isolates), *S. hyalinum* (three isolates), *T. rubrum* (two isolates), *T. interdigitale* (two isolates), *C. albicans* (two isolates) and *A. fumigatus* (two isolates). All cultures originated from human infections. Each isolate was inoculated onto two slants of Sabouraud's dextrose agar in test-tubes and incubated for 1 week at 28°C. 7·5 ml of 1:5000 thiomersal was then added to each tube and these were incubated for 24 h at 28°C. The supernatants were then removed, membrane filtered and concentrated 30–50 fold in Amicon B-15 macrosolute concentrators (Amicon Ltd, Stonehouse, Glos., U.K.).

### *Antisera*

*Patients' sera.* Sera from 35 patients with an active non-dermatophyte infection (*H. toruloidea* $n=26$; *S. hyalinum* $n=6$; *H. toruloidea* and *S. hyalinum*, $n=3$) were tested against all the cytoplasmic antigens. Six of these patients had a concurrent infection with a dermatophyte (*T. rubrum* $n=5$; *T. interdigitale* $n=1$) and four had experienced a previous infection with a dermatophyte. These test sera were compared with sera from a control group of 35 patients comprising 20 patients with active dermatophytosis who had been born outside the U.K., 10 immigrant patients without active fungal infections, and five members of the laboratory staff who had been exposed to cultures and scrapings from non-dermatophyte patients. All human sera were concentrated five-fold before testing using Amicon B 125 macrosolute concentrators.

*Rabbit antisera.* Antisera to cytoplasmic extracts of *H. toruloidea*, *S. hyalinum*, *T. rubrum* and *T. interdigitale* were raised in rabbits using the following regime. At week 1, 5 mg of antigen in Freund's complete adjuvant (0·5 ml antigen + phosphate buffered saline: 0·5 ml adjuvant) were injected into the left thigh. At week 2, 5 mg of antigen in Freund's incomplete adjuvant were injected into the right thigh. Later, at week 10, 5 mg of antigen in Freund's incomplete adjuvant were injected subcutaneously into the back, in 10 small aliquots. The rabbit's serum was tested for the presence of antibody by immunodiffusion after a further 8–10 days. Commercial antisera to *C. albicans* (Mercia Brocades) and *A. fumigatus* (Bencard) were also used in the studies.

### *Experimental methods*

The techniques used for serological tests have been described previously (Evans, 1976; Axelsen, 1983). For studies using patients' sera, immunodiffusion or counterimmunoelectrophoresis were used. For a more detailed analysis of the antigenic composition of the fungal extracts, crossed immunoelectrophoresis was employed.

*Immunodiffusion tests.* A Shandon cutter was used to produce a well pattern with a 12 mm serum well (130 $\mu$l) and six peripheral 4 mm antigen wells (15–20 $\mu$l) with an edge separation of 6 mm. The gel was 1·5% ionagar (Oxoid Agar No: 2) in McIlvaine's citric acid/phosphate buffer pH 7·0; antigen concentrations were 20 mg/ml and 2 mg/ml (dry weight/volume), and incubation was for 72 h at 28°C.

*Counterimmunoelectrophoresis.* This was performed in 1% agarose (Miles Laboratories, Slough, Bucks, U.K.) in barbitone buffer pH 8·2. For specificity tests, 4 mm wells were cut 4 mm apart. For patients' sera a 6 mm serum well (35 $\mu$l) and a 2·5 mm antigen well (5 $\mu$l) were used. Antigen concentrations were 20 mg/ml and 2 mg/ml. Tests were run at 4 V/cm for 90–120 min. Slides were washed in 5% sodium citrate prior to processing.

*Crossed and intermediate gel immunoelectrophoresis.* In crossed immunoelectrophoresis the antigenic constituents of an extract are separated by allowing them to migrate in an electric field. A second dimension of electrophoresis is then carried out at right angles to the first and the individual antigens are precipitated as peaks in a gel containing homologous antiserum. To visualize the maximum number of peaks, a series of experiments varying the ratio of antigen to antiserum may be necessary.

The insertion of an intermediate gel containing heterologous antiserum raised to a different extract in the second dimension of electrophoreis leads to precipitation of cross-reacting antigens in the intermediate gel.

The tests were performed in 1% agarose (Litex HSB) in tris/barbiturate buffer pH 8·6 (0·44% diethyl barbituric acid, 0·88% Tris, 0·01% calcium lactate). Antigen concentrations were *H. toruloidea* 67 mg/ml and *S. hyalinum* 50 mg/ml. The volume of antigen per slide varied from 5 μl to 20 μl. The serum volume per slide (56 mm × 50 mm area) ranged from 150 μl to 1 ml of five-fold concentrated rabbit antiserum. The first dimension was run with cooling at 10 V/cm for 50 min. The second dimension was run uncooled at 1·5 V/cm overnight.

*Fused rocket immunoelectrophoresis*. Fused rocket immunoelectrophoresis allows the comparison of a considerable number of samples on one slide. Samples are placed in a series of wells and driven into an antiserum containing gel. The number of precipitin lines formed by each sample can be counted, and fusion of adjacent lines indicates cross reactivity.

The tests were performed in 1% agarose (Litex HSB) in tris/barbiturate buffer at pH 8·6. Exoantigens and control cytoplasmic antigens were applied in 2·5 mm wells (5 μl) and run into two concentrations of rabbit antiserum in an area of 84 × 74 mm. The experiment was run uncooled overnight at 1·5 V/cm.

*Staining*. All gels were washed in several changes of 0·4% sodium chloride + 0·1% sodium azide for 48–72 h, dried and stained with Coomassie Brilliant Blue R (Sigma Diagnostic, Poole, Dorset, U.K.).

## RESULTS

### *Patients' sera*

Twelve (34%) of the patients with *H. toruloidea* or *S. hyalinum* infections had precipitins to HT or SH antigens detected by immunodiffusion ($n = 9$) or counterimmunoelectrophoresis ($n = 11$) (Fig. 1), compared with only three (9%) of the control group ($P > 0{\cdot}01$, $\chi^2$ test). Nine (26%) of the patients with a non-dermatophyte infection had antibodies to dermatophyte antigen (TR or TI) compared with 13 (37%) of the control group.

### *Specificity tests using hyperimmune animal antisera*

*Cytoplasmic extracts*. The results of both immunodiffusion tests and countercurrent immunoelectrophoresis are shown in Table 1. There was extensive cross-reactivity between

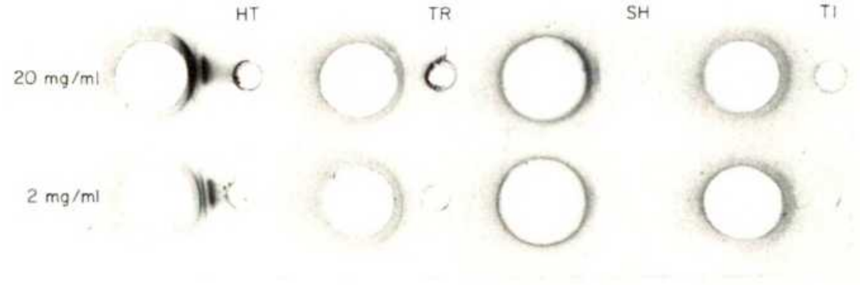

FIGURE 1. Countercurrent-immunoelectrophoresis. 6 mm serum wells contain 35 μl concentrated patient serum; 2·5 mm wells contain 5 μl cytoplasmic extract at 20 mg/ml and 2 mg/ml. Precipitin lines are visible with *H. toruloidea* and *S. hyalinum* antigens. HT, *H. toruloidea*; TR, *T. rubrum*; SH, *S. hyalinum*; TI, *T. interdigitale*.

TABLE 1. Specificity of *H. toruloidea* and *S. hyalinum* antisera/cytoplasmic antigens

| Cytoplasmic antigens | Hyperimmune animal antisera | | | | | |
|---|---|---|---|---|---|---|
| | *H. toruloidea* | *S. hyalinum* | *T. rubrum* | *T. interdigitale* | *C. albicans* | *A. fumigatus* |
| *H. toruloidea* | + + + | + + + | − | − | − | − |
| *S. hyalinum* | + + + | + + + | − | − | − | − |
| *T. rubrum* | − | − | + + + | + | − | − |
| *T. interdigitale* | − | − | + + | + + + | − | − |
| *C. albicans* | − | − | − | − | + + + | − |
| *A. fumigatus* | − | − | − | − | − | + + + |

+ + + = Strong; + + = moderate; + = weak; − = no reaction.

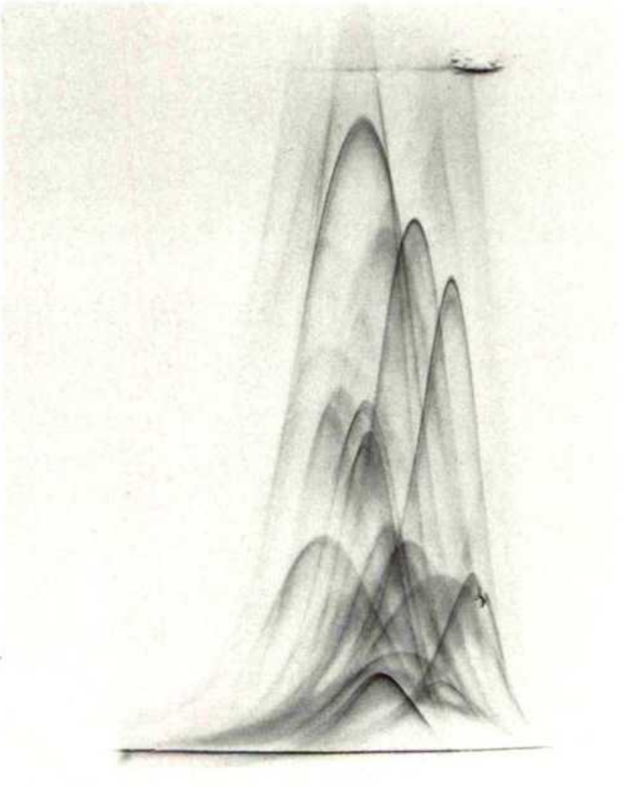

FIGURE 2. Crossed-immunoelectrophoresis—*H. toruloidea*. 20 μl *H. toruloidea* cytoplasmic extract was separated in a first dimension run at 10 V/cm for 50 min; the second dimension into a gel containing 150 μl concentrated homologous antiserum was run at 1·5 V/cm overnight. 25 of the total 34 peaks detected are visible in this slide.

*H. toruloidea* and *S. hyalinum* antigens and antisera. The two dermatophyte species tested (*T. rubrum* and *T. interdigitale*) also cross-reacted with each other.

The cross-reactivity between *H. toruloidea* and *S. hyalinum* was examined in more detail. Crossed immunoelectrophoresis was performed using homologous antigens and antisera at various concentrations and the pattern and number of antigen peaks for the two extracts were determined.

Thirty-four peaks were detected in the *H. toruloidea* system (Fig. 2) and 41 peaks in the *S. hyalinum* system (Fig. 3). Intermediate gel immunoelectrophoresis was then performed to determine the number of common antigens between the systems. When *H. toruloidea* cytoplasmic extract was run into homologous antiserum through an intermediate gel containing antiserum to *S. hyalinum*, at least 21 antigens (total 26) were seen to cross-react with the heterologous antiserum (Fig. 4). Conversely, when *S. hyalinum* was run into its homologous

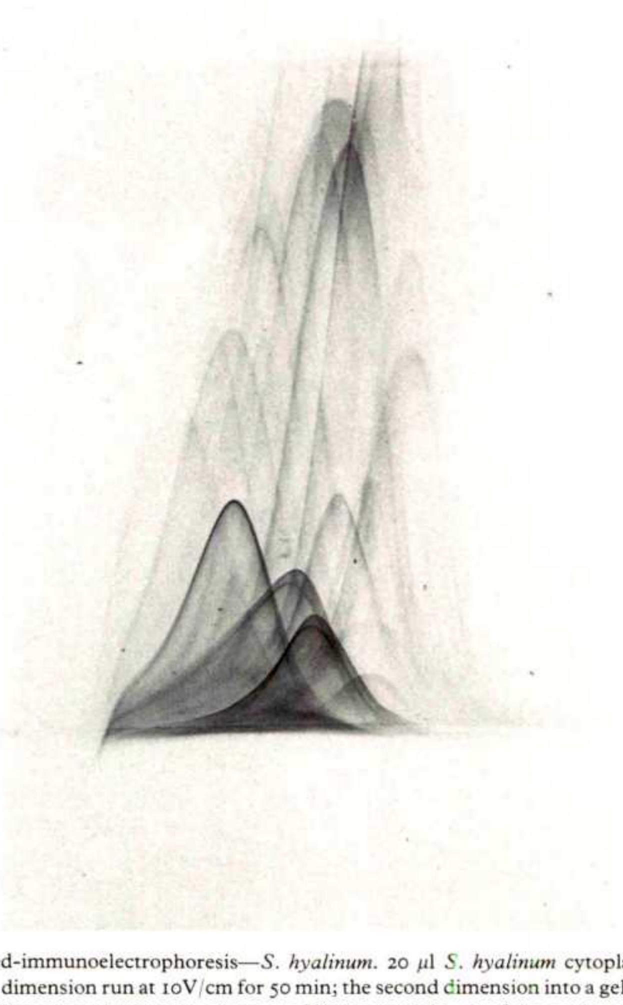

FIGURE 3. Crossed-immunoelectrophoresis—*S. hyalinum*. 20 μl *S. hyalinum* cytoplasmic extract was separated in a first dimension run at 10V/cm for 50 min; the second dimension into a gel containing 400 μl concentrated homologous antiserum was run at 1·5 V/cm overnight. 30 of the total 41 peaks detected are visible in this slide.

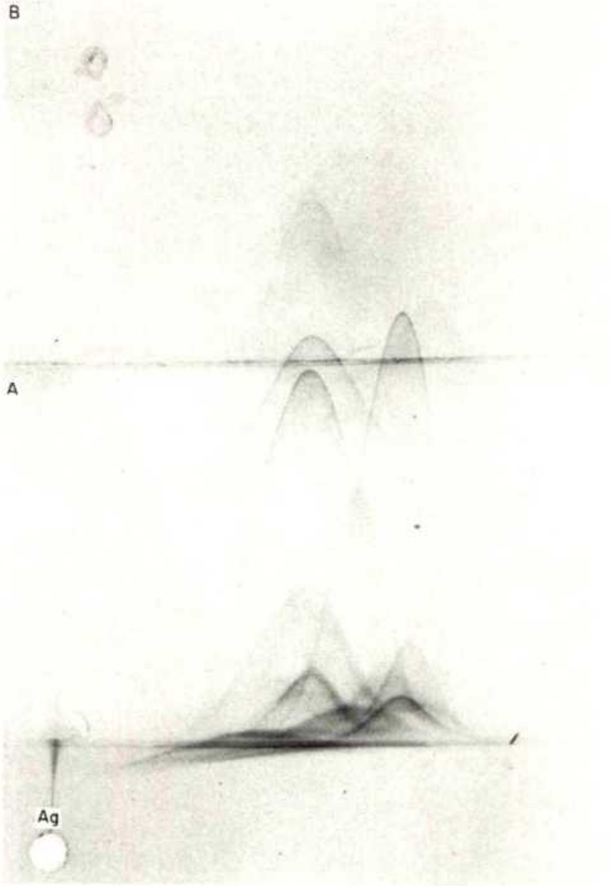

FIGURE 4. Intermediate gel immunoelectrophoresis. 5 μl *H. toruloidea* cytoplasmic extract was separated at 15 V/cm for 50 min. The second dimension run was through a gel containing 250 μl concentrated rabbit antiserum raised to *S. hyalinum* cytoplasmic extract (A) into a gel containing 115 μl of homologous antiserum (B). Ag, antigen well. 21 antigens showed cross-reactivity with the heterologous serum.

antiserum through an intermediate gel containing *H. toruloidea* antiserum, at least 33 antigens cross-reacted with the heterologous antiserum (total 39) (Fig. 5).

*Exoantigens*. Cross-reactivity between *H. toruloidea* and *S. hyalinum* exoantigens was demonstrated both in immunodiffusion tests and in fused rocket immunoelectrophoresis tests (Table 2); again, no cross-reactions were detected with *T. rubrum*, *T. interdigitale* or *C. albicans*, but weak cross-reactivity was demonstrated between *A. fumigatus* exoantigens and *H. toruloidea* rabbit antiserum in rocket immunoelectrophoresis slides (Fig. 6). The sensitivity of the rocket immunoelectrophoresis tests also allowed the demonstration of a relatively large number of antigens in the exoantigen preparations from *H. toruloidea* and *S. hyalinum* isolates. Although the method of preparation is simple and quick, over 15 peaks could be counted in some rockets, which represent a considerable proportion of those revealed in the cytoplasmic extracts on crossed electrophoresis using the same antiserum.

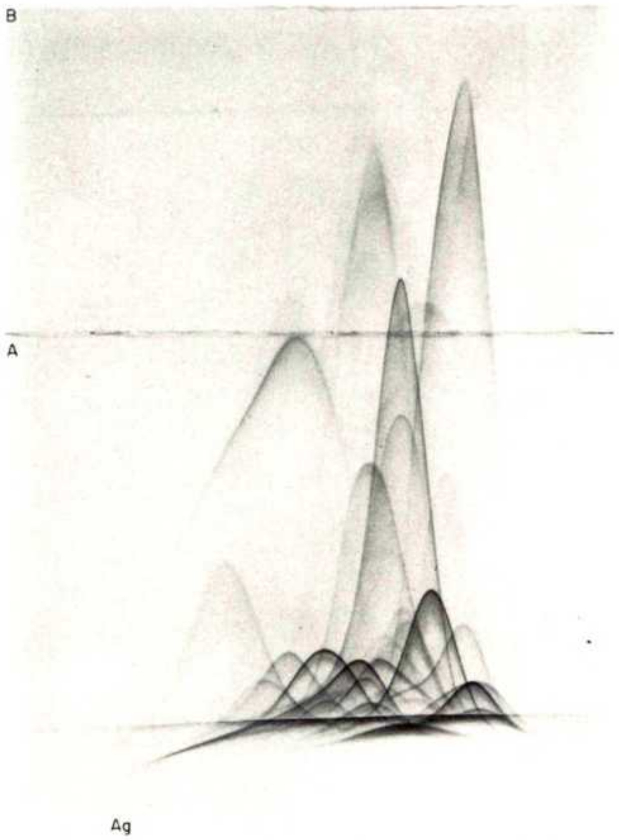

FIGURE 5. Intermediate gel immunoelectrophoresis. 5μl *S. hyalinum* cytoplasmic extract was separated at 10 V/cm for 50 min. The second dimension run was through a gel containing 200 μl concentrated rabbit antiserum raised to *H. toruloidea* cytoplasmic extract (A) into a gel containing 200 μl concentrated homologous antiserum (B). Ag, antigen well. At least 33 antigens showed cross-reactivity with the heterologous serum.

TABLE 2. Specificity of exoantigen tests

| Exoantigens | No. of isolates | Antisera to cytoplasmic antigens (no. of positive tests) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *H. toruloidea* | *S. hyalinum* | *T. rubrum* | *T. interdigitale* | *C. albicans* | *A. fumigatus* |
| *H. toruloidea* | | | | | | | |
| Form 1 | 3 | 3 | 3 | – | – | – | – |
| Form 2 | 3 | 3 | 3 | – | – | – | – |
| Form 3 | 3 | 3 | 3 | – | – | – | – |
| *S. hyalinum* | 3 | 3 | 3 | – | – | – | – |
| *T. rubrum* | 2 | – | – | 2 | – | – | – |
| *T. interdigitale* | 2 | – | – | – | 2 | – | – |
| *C. albicans* | 2 | – | – | – | – | 2 | – |
| *A. fumigatus* | 2 | 2* | – | – | – | – | 2 |

* Trace reaction only.

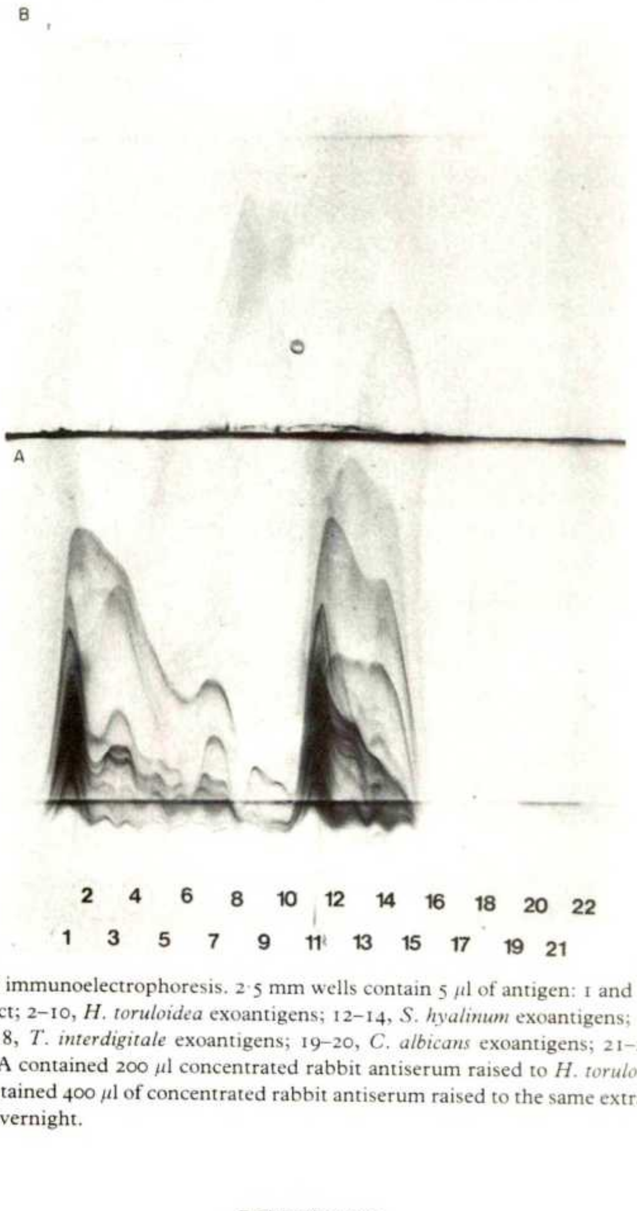

FIGURE 6. Rocket immunoelectrophoresis. 2·5 mm wells contain 5 μl of antigen: 1 and 11, *H. toruloidea* cytoplasmic extract; 2–10, *H. toruloidea* exoantigens; 12–14, *S. hyalinum* exoantigens; 15–16 *T. rubrum* exoantigens; 17–18, *T. interdigitale* exoantigens; 19–20, *C. albicans* exoantigens; 21–22, *A. fumigatus* exoantigens. Gel A contained 200 μl concentrated rabbit antiserum raised to *H. toruloidea* cytoplasmic extract. Gel B contained 400 μl of concentrated rabbit antiserum raised to the same extract. The test was run at 1·5 V/cm overnight.

## DISCUSSION

A significant proportion (34%) of the patients with *H. toruloidea* or *S. hyalinum* infections examined had antibodies to the fungi compared with non-infected controls. The proportion of patients with positive serological responses detected was similar to that seen in *T. rubrum* infections of the palms and soles using counterimmunoelectrophoresis and a similar method of antigen preparation (Hay & Shennan, 1982). The incidence and titre of positive antibody responses in dermatophytosis vary with a number of factors, including the identity of the infecting organism, the site of infection, the sensitivity of the serological tests and the type of antigen used. For instance, using similar immunological methods, widespread or chronic infections caused by other dermatophyte species such as *Trichophyton schoenleinii* (Grappel, Blank & Bishop, 1974) or *Trichophyton concentricum* (Hay & Shennan, 1984) are associated with a much higher level of positive antibody responses, while in dermatophytosis caused by *T. rubrum* the site of infection may affect the proportion of positive reactions with palmar or plantar dermatophytosis producing the lowest number of responses (Attapattu & Clayton, 1982). If a highly sensitive method of antibody detection such as enzyme-linked immunosorbent assay (ELISA) is employed, even non-infected controls may show antibody to dermatophytes (Sohnle, Collins-Lech & Muhta, 1983). Our results in patients with *H. toruloidea* or *S. hyalinum* infections are also likely to be affected by similar variables, particularly the serological tests used and the localization of infection in the thick stratum corneum.

While the presence of antibodies in infected patients indicated the development of an immune response even in the absence of marked inflammation, their role in host defence against *S. hyalinum* and *H. toruloidea* is unknown. In dermatophytosis, specific antibodies do not appear to contribute significantly to recovery, and the development of T lymphocyte-mediated immune responses appears to be the critical event (Calderon & Hay, 1984). However, in dermatophyte infections the presence of antibodies may affect the pathogenesis of the disease in a number of different ways, ranging from blocking of T lymphocyte-mediated responses and cross-reactions with epidermal intracellular substance (Hopper, Grappel & Blank, 1975) to the development of immediate type hypersensitivity during infection (Jones, Reinhardt & Rinaldi, 1974). These actions may influence the course and expression of symptoms during infection. It remains to be seen whether antibodies to *H. toruloidea* or *S. hyalinum* show similar activity.

This study has also shown that antigens of *H. toruloidea* and *S. hyalinum* prepared by two different methods, cytoplasmic extraction and extra-cellular diffusion (exoantigens), are very similar. Both antigens are complex and have a large number of components which cross-react. They are also substantially distinct from similar antigens prepared from dermatophytes, *C. albicans* or *A. fumigatus*.

Fused rocket immunoelectrophoresis shows that, while there are quantitative differences in antigen composition of the three morphological variants of *H. toruloidea*, they are qualitatively very similar. It is not possible from this work to show that *H. toruloidea* and *S. hyalinum* are antigenically identical but they share so many common antigens that they would appear to be closely related.

There are three main practical consequences of the detailed antigenic analysis of *H. toruloidea* and *S. hyalinum*. Firstly, because they are antigenically distinct it is possible to use their production of diffusable antigens (exoantigens) as a means of confirming the identity of cultures. Such techniques have already been used successfully for the identification of other fungi which are either atypical or hazardous (Standard & Kaufman, 1976). Secondly, the presence of *S. hyalinum* and *H. toruloidea* in tissue or skin scrapings could be detected by indirect immunofluorescence or immunoperoxidase techniques (Chandler, Kaplan & Ajello, 1980). Hyphae of the organisms could also be distinguished from those of dermatophytes in mixed infections, using similar methods. Finally, it is now possible to study other aspects of the specific immune response to fungal antigens, including T lymphocyte activity in patients with *H. toruloidea* or *S. hyalinum infections*.

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# HLA antigens and Langerhans cell density in contact dermatitis

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## SUMMARY

We studied 67 patients with multiple contact allergies to determine whether there was an association of this state with any particular HLA antigen. HLA-A, -B and DR antigens were typed by standard serological methods. There was no significant HLA association, although there was an increased frequency of DR4 in those patients who included nickel as one of their sensitivities (64% compared with 33% in controls), and an increase in DR6 in those patients who included sensitivity to a rubber accelerator (45% compared with 16% in controls). However, when the probabilities were corrected for the number of HLA antigens tested and the number of substances in the patch test battery, these associations were no longer statistically significant. We also examined the morphology and numbers of Langerhans cells in epidermal sheets from six subjects with multiple allergies. There were no differences in appearance or numbers of Langerhans cells stained for ATPase, compared with 20 non-allergic controls.

Allergic contact dermatitis is an inflammatory reaction mediated by type IV hypersensitivity (Gell, Coombs & Ladiron, 1975). In laboratory animals, genes controlling the delayed hypersensitivity reaction have been linked to those controlling the major histocompatibility antigens (McDevitt & Benaceraff, 1969). The immune response genes (Ia) of mice have their homologues in the human HLA-DR locus. The DR antigens are found only on cells capable of eliciting the mixed lymphocyte culture reaction (Duvic & Goldsmith, 1983), and are present on a restricted range of cells, including B-lymphocytes, macrophages, epithelial cells, Langerhans cells and other activated, blastoid T-lymphocytes (but not small T-lymphocytes) (Shackleford *et al.*, 1982).

Patients with multiple allergies, defined by the presence of unequivocal positive reactions to three or more substances from the European Standard Battery, have been shown to have an enhanced susceptibility to sensitization (Moss, Friedmann & Shuster, 1985). We have investigated whether this susceptibility is linked to any particular HLA-DR allele.

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Epidermal Langerhans cells are important in the induction of allergic contact sensitization (Stingl & Wolff, 1983). We also investigated whether there was any abnormality of Langerhans cell number or morphology in the skin of patients with multiple contact allergies whose skin condition was quiescent.

## METHODS

### *Patients with multiple allergies*

Sixty-seven patients from the Royal Victoria Infirmary patch test clinic were studied. These patients had unequivocal positive reactions to three or more of the allergens present in the European Standard Battery. Patients with industrial exposure and patients with exposure to multiple medicaments were excluded.

### *Nickel allergy*

Twenty patients were tested who were sensitive to nickel alone.

### *HLA typing*

The lymphocytes for HLA typing were separated from heparinized whole blood using dextran and carbonyl iron sedimentation followed by layering over Ficoll-isopaque solution. B cells for DR typing were separated using the sheep cell rosetting technique of Gelsthorpe and Doughty (1977).

A complement-dependent lymphocytotoxicity test, a modification of the technique suggested by Mittal *et al.* (1968), was used to test the patients' lymphocytes against at least 60 individual HLA-A and -B antisera, and 60 DR antisera, with several sera to cover each 'broad' specificity.

The control group consisted of healthy subjects (blood donors) taken from the same geographical area as the test patients. Five hundred subjects had been typed for HLA-A and -B antigens and 245 of these had also been typed for DR antigens.

The statistical analysis of the data was performed by using a chi squared test and corrected for the number of antigens tested. Rather than refer observed chi squared values to a chi squared distribution, because eight DR antigens were tested, the distribution of the largest values in eight sets of randomly generated independant chi squared values was calculated. The *P* values quoted refer to this distribution.

### *Langerhans cells*

Epidermis was obtained from suction blisters from six patients with multiple contact allergies and 20 healthy controls. Langerhans cells were stained for ATPase (Wolff & Winkelmann, 1967). Full thickness epidermal sheets were examined on a Zeiss microscope with a calibrated eyepiece graticule. Clearly visible ATPase positive cell bodies with dendrites present were counted in four graticule fields per epidermal sheet and expressed as the mean number of Langerhans cells (± SEM) per $mm^2$ of skin surface.

## RESULTS

In the multiple allergy group as a whole the frequency of HLA-A, or -B antigens or of DR antigens (Table 1) was not significantly different from the control group.

When each allergen in the standard battery was looked at in turn, in the majority of cases the numbers were too small for adequate analysis. However, in 36 patients whose sensitivities

TABLE 1. Frequency of HLA-DR antigens in 67 multiple allergy patients and 245 non-allergic controls

| | Patients | | Controls | |
|---|---|---|---|---|
| | No. | % | No. | % |
| DR1 | 9 | 13 | 24 | 10 |
| DR2 | 17 | 25 | 71 | 29 |
| DR3 | 17 | 25 | 74 | 30 |
| DR4 | 29 | 44 | 81 | 33 |
| DR5 | 7 | 10 | 26 | 11 |
| DR6 | 15 | 23 | 40 | 16 |
| DR7 | 17 | 25 | 55 | 22 |
| DR8 | 2 | 3 | 8 | 3 |

TABLE 2. Frequency of HLA-DR antigens in 36 multiple allergy patients whose sensitivities included nickel

| | Patients | | Controls |
|---|---|---|---|
| | No. | % | % |
| DR1 | 4 | 11 | 10 |
| DR2 | 7 | 19 | 29 |
| DR3 | 9 | 25 | 30 |
| DR4 | 23 | 64 | 33 |
| DR5 | 1 | 3 | 11 |
| DR6 | 7 | 19 | 16 |
| DR7 | 12 | 33 | 22 |
| DR8 | 2 | 6 | 3 |

included nickel, 23 had antigen DR4 (64%) compared with 33% in the control group ($P = 0{\cdot}005$) (Table 2), while for those 20 patients whose sensitivities included one of the rubber accelerators (Thiuram, mercapto mix or mercaptobenzothiazole), nine had antigen DR6 (45%) compared with 16% in the control group ($P < 0{\cdot}02$) (Table 3). When corrected for the number of HLA antigens tested (Katz, 1977), the group with nickel as one of their sensitivities still showed a significant increase in the frequency of DR4 ($P < 0{\cdot}05$), but the group which included the rubber accelerators no longer showed a significantly increased frequency of DR6 ($P > 0{\cdot}1$). As there are 22 substances in the European standard battery, and we looked for an association with each one, this also needed to be corrected for. When this was done, the result in the multiple allergy patients who were sensitive to nickel was no longer significant. The frequency of DR4 in patients sensitive to nickel only was 8 out of 20 (40%) (Table 4). There was no significant difference between these patients and healthy controls, and no significant difference in the frequency of DR4 between patients sensitive only to nickel and those with nickel as one of their multiple sensitivities.

TABLE 3. Frequency of HLA-DR antigens in 20 multiple allergy patients whose sensitivities included a rubber accelerator

| | Patients | | Controls |
|---|---|---|---|
| | No. | % | % |
| DR1 | 2 | 10 | 10 |
| DR2 | 2 | 10 | 29 |
| DR3 | 5 | 25 | 30 |
| DR4 | 9 | 45 | 33 |
| DR5 | 3 | 15 | 11 |
| DR6 | 9 | 45 | 16 |
| DR7 | 4 | 20 | 22 |
| DR8 | 0 | 0 | 3 |

TABLE 4. Frequency of HLA-DR antigens in 20 patients sensitive to nickel only

| | Patients | | Controls |
|---|---|---|---|
| | No. | % | % |
| DR1 | 2 | 10 | 10 |
| DR2 | 5 | 25 | 29 |
| DR3 | 6 | 30 | 30 |
| DR4 | 8 | 40 | 33 |
| DR5 | 2 | 10 | 11 |
| DR6 | 6 | 30 | 16 |
| DR7 | 6 | 30 | 22 |
| DR8 | 0 | 0 | 3 |

The morphology and number of ATPase-stained Langerhans cells in the multiple allergy group did not differ from the controls (739 ± 22 cells/mm$^2$ and 720 ± 60 cells/mm$^2$ in the allergy and control groups respectively).

## DISCUSSION

Our findings indicate that people with an increased susceptibility to the development of contact hypersensitivities are a heterogeneous group, not characterized by the presence of any particular HLA type. Other workers have also examined the distribution of HLA antigens in subjects with contact allergy. Roupe, Rydberg and Swanbeck (1979) and Valsecchi *et al.* (1982) did not find any association of HLA-A, -B or -C antigens in patients with contact dermatitis. Liden *et al.* (1978) found nine associations involving six different HLA antigens and six different allergens. This was in a group of patients with single and multiple allergies and the results were not corrected for the number of antigens or allergens. Two of these teams (Roupe *et al.*, 1979; Valsecchi *et al.*, 1982) postulated an association between HLA-DR antigens and contact dermatitis.

It is reasonable to suppose that such an association might exist from the evidence in animals that the genes controlling immune responses are linked to the major histocompatibility complex. However, such an association is potentially complex since there are different levels at which immune responses may be under genetic control. Firstly, there is the level of general capacity to respond—whether high or low, which is non-antigen specific. Secondly, there is the control of responses to individual antigens.

Although in the group as a whole there was no association with any HLA-DR type, there was an association of HLA DR6 with the presence of sensitivity to 'rubber accelerators'. However, when the necessary corrections were made for the number of HLA antigens examined ($n=46$) and the number of contact allergens ($n=22$), this association was no longer statistically significant. Perhaps more importantly, there was an increase of DR4 in subjects with nickel among their sensitivities. The prevalence was 64% compared with 33% of non-allergic controls. This increase was not present in subjects sensitive to nickel alone. Once again, when corrected for the number of HLA antigens tested and the number of allergens in the standard battery, this increase was not statistically significant.

Another determinant of contact sensitization is the function of epidermal Langerhans cells. The number and functions of Langerhans cells may be impaired by the action of ultraviolet radiation, PUVA and topical glucocorticoids. Although the converse state of augmented number and function has yet to be discovered, it seems logical that it might pertain to subjects with increased susceptibility to contact sensitization. We found no obvious abnormality in the numbers or morphology of Langerhans cells. However, determination of their functional competence would require a different type of experimental design.

We conclude that people with a tendency to develop multiple contact allergies are a genetically heterogeneous group with no common HLA antigen association or gross abnormality of Langerhans cells. It seems likely, therefore, that they represent the high responder end of the normal distribution.

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# A quantitative study of the effect of topical indomethacin on cutaneous erythema induced by UVB and UVC radiation

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## SUMMARY

The effect of indomethacin gel on UVB and UVC erythema was assessed objectively using a reflectance instrument. When indomethacin was applied immediately after irradiation, UVR (ultraviolet radiation) dose-dependent suppression of erythema was demonstrated for both wavelengths until 36 h after irradiation when both indomethacin and control gel base-treated sites were equally erythematous. Suppression of erythema also occurred when application of indomethacin was delayed until 24 h after irradiation, showing that for both wavelengths prostaglandin synthesis remains increased throughout this period. The degree of suppression at any time, however, was no greater than that achieved by a single application immediately after irradiation, indicating that the eventual equal erythema of indomethacin and gel base-treated sites was not due to tachyphylaxis or inadequate dosage.

Construction of dose-response curves for the indomethacin-responsive and indomethacin-unresponsive components of erythema shows that in human skin the difference in erythemal response to UVB and UVC radiation is not due to the formation of different mediators at these wavelengths.

Cyclo-oxygenase products of arachidonic acid metabolism have been implicated in the inflammatory response to both UVB and UVC radiation (Mathur & Gandhi, 1972; Black *et al.*, 1978a; Camp *et al.*, 1978). Indomethacin, a potent inhibitor of the enzyme prostaglandin synthetase, is known to reduce the intensity of UVB-induced erythema when given topically (Snyder & Eaglstein, 1974a), intradermally (Snyder & Eaglstein, 1974b), or orally (Black *et al.*, 1978b). UVC-induced erythema is also inhibited by oral indomethacin (Black *et al.*, 1978b); however, Eaglstein and Marsico (1975) found that the dose of intradermal indomethacin required for inhibition was considerably higher for UVC than for UVB erythema, and suggested that different inflammatory mediators were operating at these two wavelengths.

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The development of a reflectance instrument to quantify erythema (Diffey, Oliver & Farr, 1984) has allowed dose-response curves to be constructed for UVR of different wavelengths (Farr & Diffey, 1985). The slope of the linear portion of the UVR-(log dose) erythema-response curve is significantly greater for 300 nm (UVB) radiation than for 254 nm (UVC) radiation, and this suggests that different mediators, or mechanisms of mediator release, may be involved in the causation of erythema at these two wavelengths. In order to investigate further the mechanism of ultraviolet erythema, the response of UVB and UVC erythema to topical indomethacin has been measured objectively for different doses of UVR, with regard to amount of indomethacin applied, time of application after irradiation, and time of observation.

## METHODS

### *Subjects*

Twenty-eight adult volunteers (sun-reactive skin type II or III) were studied. No subject was known to exhibit abnormal sensitivity to sunlight, or was taking any medication known to provoke such a reaction. Thirteen of the volunteers had normal skin and 15 were being investigated or treated for localized skin disease. In all cases, skin of the mid-back was studied and all measurements were made on skin of normal clinical appearance. No topical treatment, other than indomethacin gel (or gel base) was applied to any part of the back before or during the study.

### *Photo-irradiation apparatus and radiation dosimetry*

Two optical radiation sources were used: (a) a germicidal lamp (Philips type TUV 15W) for irradiation at 254 nm, and (b) a medium pressure mercury arc lamp (Philips type CS 100W) in conjunction with Schott WG305 and UG5 colour glass filters; this combination results in a spectrum where approximately 90% of the erythemally effective power lies in the 297 and 302 nm characteristic lines, with the remaining 10% lying chiefly in the 313 nm line. Radiation from this source is referred to as 300 nm radiation.

### *Measurement of erythema*

Erythema was measured using a reflectance instrument which obtains an 'erythema index' related to the blood content of the superficial dermis (Diffey *et al.*, 1984). Before irradiation, three measurements of the erythema index were made at each site, with the subject lying prone on a couch. The measurements of erythema were repeated at various times after irradiation, and the increase in erythema calculated as the difference between the mean post- and mean pre-irradiation erythema index at each site (Farr & Diffey, 1984).

### *Application of indomethacin*

A measured volume of indomethacin gel 1% (w/v) in a hydroalcohol base (or gel base alone) was applied to a marked area of skin, spread to approximately even thickness using a gloved finger, and allowed to dry.

### *Response to varying doses of indomethacin gel*

Seven sites were exposed to a fixed dose of UVR (five subjects at 300 nm; five subjects at 254 nm), sufficient to cause moderate erythema (1·0–1·5 kJ/m$^2$ at 300 nm; 2·0–2·5 kJ/m$^2$ at 254 nm). Immediately after irradiation, different volumes of gel were applied to five sites to achieve a surface density of indomethacin varying from 12·5 to 200 $\mu$g/cm$^2$. The gel base was applied to one site (0·1 ml to 10 cm$^2$), and the remaining site was left untreated. Erythema was measured 8 and 24 h after irradiation.

### *UVR dose-response*

Six closely apposed circular sites on either side of the back were irradiated consecutively using a series of exposure doses increasing geometrically. The dose increment factor was $\sqrt{2}$ for 300 nm radiation and approximately 2 for 254 nm radiation. The maximum exposure dose given to any one subject varied from 1·52 to 1·92 kJ/m$^2$ at 300 nm and from 2·33 to 2·88 kJ/m$^2$ at 254 nm. On each side of the back, one site was not irradiated and served as a control. In five subjects at each wavelength, indomethacin gel (100 μg/cm$^2$) was applied immediately after irradiation and subsequently at 12, 24 and 36 h after irradiation to the sites on one side of the back. An equivalent volume of gel base was applied at the same times to the sites on the other side of the back. In three subjects at each wavelength, indomethacin (or gel base) was also applied 4 h before irradiation. Erythema was measured at each site prior to irradiation and at 4, 8, 12, 36 and 48 h after irradiation. Between 7 and 14 days after irradiation, in 13 of the subjects on whom UVR dose-response studies were performed, the degree of melanin pigmentation was compared visually at the indomethacin and gel base-treated sites.

### *Delayed application of indomethacin after irradiation*

In three subjects at each wavelength, seven sites were exposed to a fixed dose of UVR, sufficient to cause moderate erythema (1·0–1·5 kJ/m$^2$ at 300 nm; 2·0–2·5 kJ/m$^2$ at 254 nm). Indomethacin gel (100 μg/cm$^2$) was applied to one site immediately after irradiation. Further sites were treated similarly with a single application of indomethacin at 4, 12, 24, 36 or 48 h after irradiation. One site was irradiated but left untreated. Erythema was measured before irradiation, before application of indomethacin to each of the sites, and 4 and 8 h after application of indomethacin. In a further two subjects at each wavelength, the speed of response of moderate erythema to indomethacin (100 μg/cm$^2$), or an equivalent volume of gel base, applied 12 h after irradiation was determined. Erythema was measured at hourly intervals until 8 h after application of indomethacin.

### *Statistical analysis of results*

The significance of results on single subjects was tested using a two-sample *t*-test, and the variance ratio (*F* test) was used for results based on groups of subjects.

## RESULTS

### *Response to varying doses of indomethacin gel*

Indomethacin caused inhibition of both 254 and 300 nm erythema measured 8 and 24 h after irradiation. The degree of inhibition was dependent on the surface density of indomethacin and was similar for both wavelengths (Fig. 1). Significant reduction in erythema occurred at both times of measurement with the lowest dose of indomethacin (12·5 μg/cm$^2$) ($P < 0·01$, *t*-test). In all subjects, for both wavelengths and at both times of measurement, maximum reduction in erythema was achieved with 100 μg/cm$^2$. No significant difference in measured erythema was found between untreated control sites and those treated with gel base.

### *UVR dose-response*

The effect of indomethacin or gel base, applied immediately after irradiation and subsequently

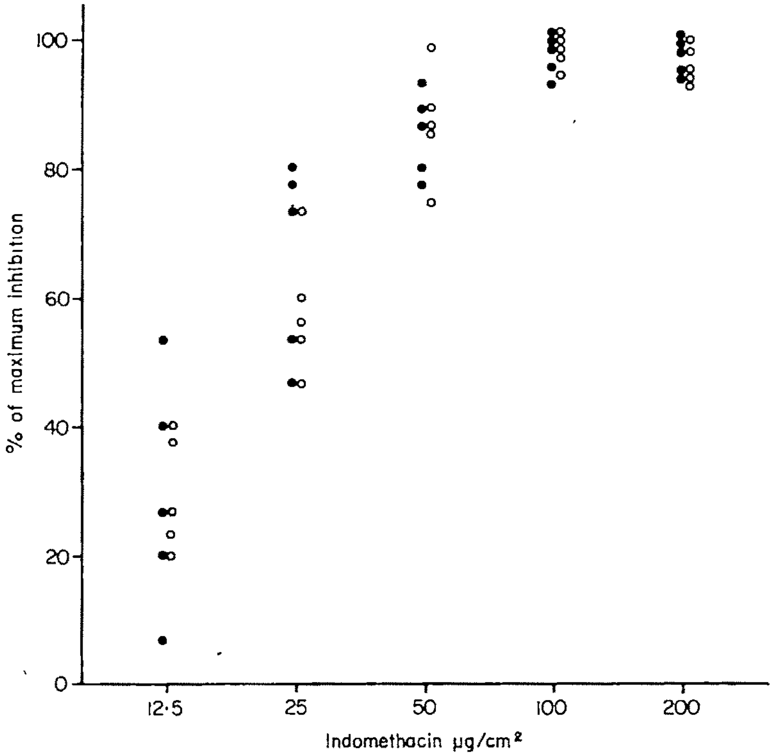

FIGURE 1. The degree of inhibition at 8 h, expressed as a percentage of maximum inhibition achieved, by different amounts of indomethacin applied immediately after irradiation, on moderate erythema in five subjects at 254 nm (○) and five subjects at 300 nm (●). The error bars have been omitted for clarity.

at 12 h intervals, on the 300 nm UVR-(log dose) erythema-response curve for one subject at different times of measurement is shown in Figure 2. Indomethacin caused a reduction in erythema from 4 to 24 h after irradiation in all subjects. The absolute reduction in measured erythema index was found to increase with the UVR exposure dose. The effect of indomethacin on the 254 nm UVR-(log dose) erythema-response curve in one subject is shown in Figure 3. As with 300 nm radiation, the absolute reduction in erythema was shown to be dependent on the UVR exposure dose. Inhibition of erythema was seen in all subjects from 8 to 24 h after irradiation.

Because of the inter-subject differences in erythemal sensitivity to UVR, results on all subjects have been grouped according to the measured increase in erythema index (ΔE) for the gel base-treated sites at 12 h after irradiation, rather than according to the exposure dose of UVR (Fig. 4). For mild to moderate 300 nm erythema (12 h $\Delta E \simeq 0{\cdot}12$, or approximately 2 MED), significant inhibition of erythema by indomethacin was seen at 4, 8, 12 and 24 h after irradiation ($P < 0{\cdot}01$, $F$ test), but not at 36 or 48 h. Significant inhibition of moderate to severe 300 nm erythema (12 h $\Delta E \simeq 0{\cdot}20$, or approximately 6 MED) persisted until 36 h ($P < 0{\cdot}05$). Indomethacin caused significant inhibition of mild to moderate 254 nm erythema (12 h $\Delta E \simeq 0{\cdot}12$, or approximately 10 MED) at 8, 12 and 24 h after irradiation ($P < 0{\cdot}01$), but not at 4, 36 or 48 h. Pre-treatment with indomethacin or gel base 4 h before irradiation did not alter the degree or time-course of inhibition at 300 nm. For 254 nm erythema, however, significant

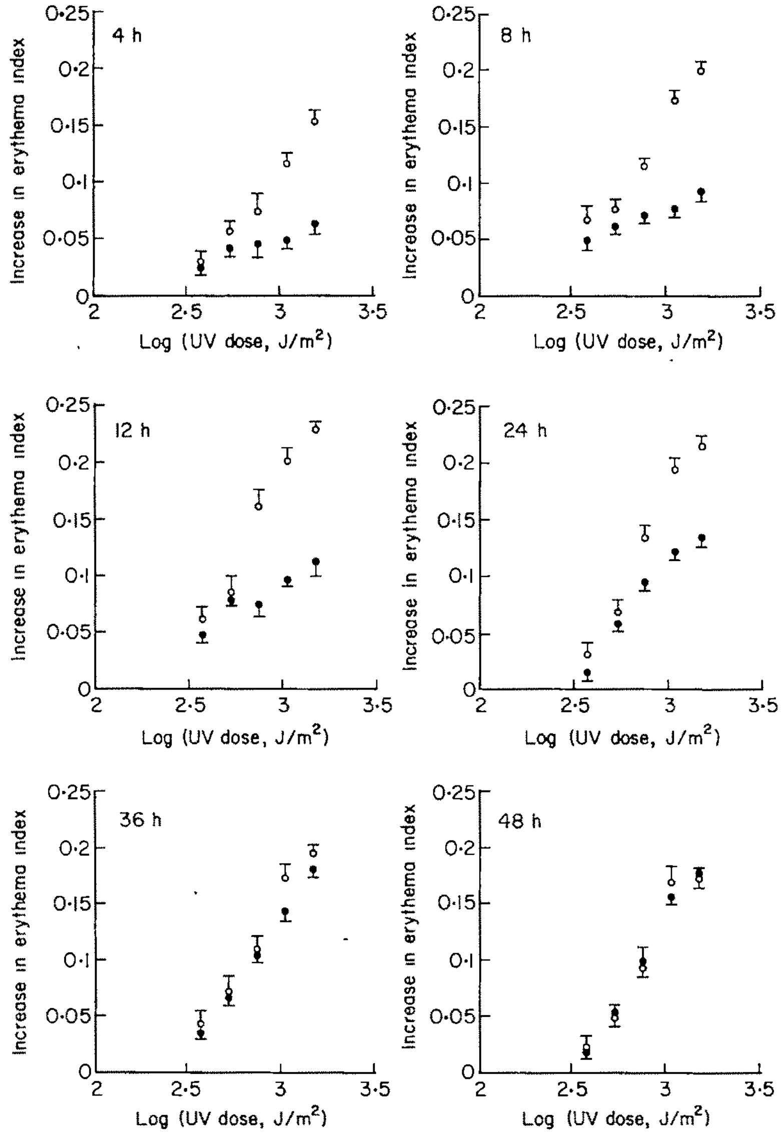

FIGURE 2. The effect of indomethacin (●) and gel base (O) on the dose-response curve for 300 nm erythema at different times of observation after irradiation in one subject. Bars represent ± 1 SD of the measured difference between the post- and pre-irradiation erythema indices.

inhibition of erythema occurred 4 h after irradiation at indomethacin pre-treated sites ($P < 0{\cdot}01$) (Fig. 4). The subsequent degree and time-course of inhibition was not affected; indomethacin and gel base-treated sites were equally erythematous at 36 and 48 h after irradiation.

Between 7 and 14 days after irradiation, increased melanin pigmentation was apparent in the majority of sites exposed to 300 nm UVR and in those exposed to the higher doses of 254 nm

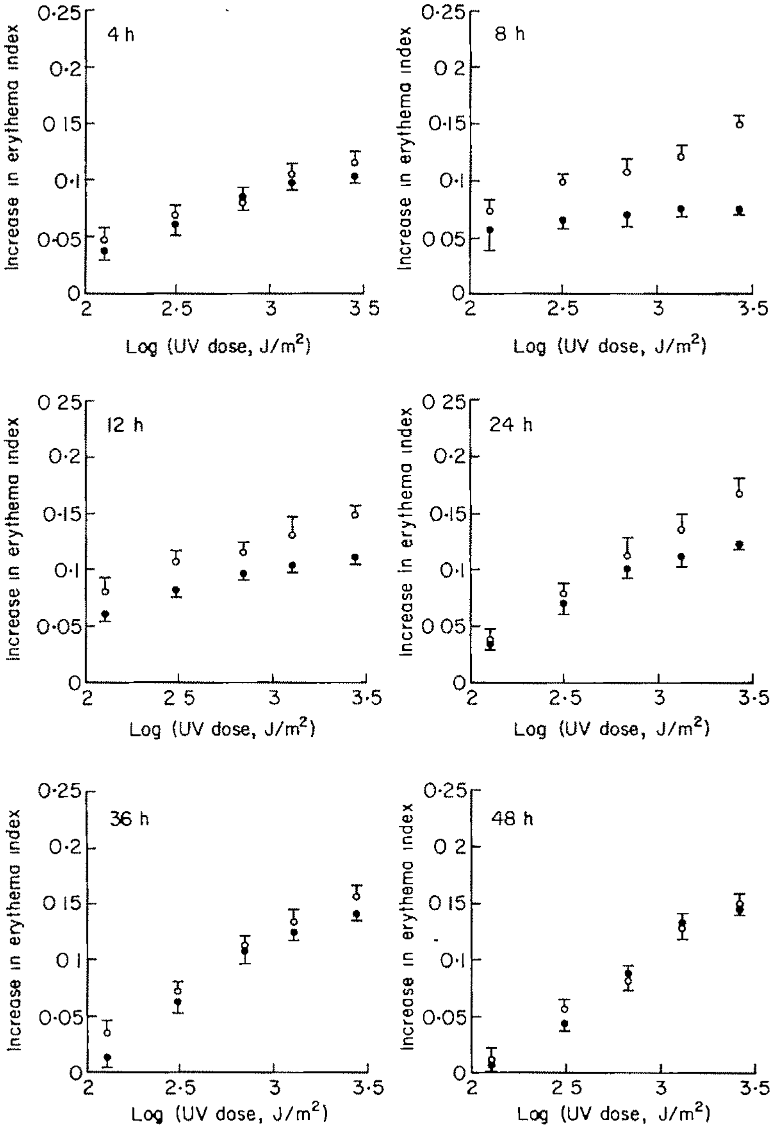

FIGURE 3. The effect of indomethacin (●) and gel base (○) on the dose-response curve for 254 nm erythema at different times of observation after irradiation in one subject. Bars represent ± 1 SD of the measured difference between the post- and pre-irradiation erythema indices

UVR. The intensity of melanin pigmentation (assessed visually) was reduced at the indomethacin-treated sites compared with those exposed to equivalent doses of UVR, and treated with gel base.

### *Delayed application of indomethacin*

In all subjects, significant inhibition of both 300 and 254 nm erythema occurred 4 to 8 h after application of indomethacin at 4, 12 or 24 h after irradiation, compared with the irradiated but

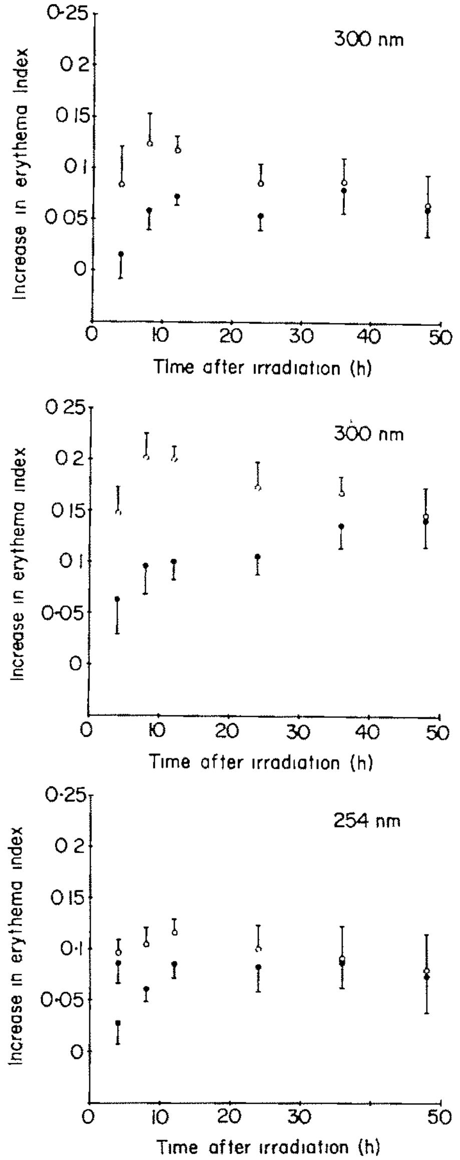

FIGURE 4. The effect of indomethacin (●) and gel base (○) on mild to moderate erythema at 300 and 254 nm, and moderate to severe erythema at 300 nm, in five subjects at different times of observation after irradiation. Bars represent ± 1 SD of the mean increase in erythema index for the five subjects at each time of observation. The effect of indomethacin application 4 h before irradiation on mild to moderate 254 nm erythema (mean increase in erythema index in three subjects, ± 1 SD) is also shown (■).

untreated sites (Fig. 5) ($P<0{\cdot}01$; $t$-test). However, irrespective of time of observation, the degree of inhibition was no greater than that achieved by a single application of indomethacin immediately after irradiation. When indomethacin was applied 36 or 48 h after irradiation, there was no significant reduction in either 300 or 254 nm erythema compared with the irradiated but untreated sites. When indomethacin was applied 12 h after irradiation, in both subjects on

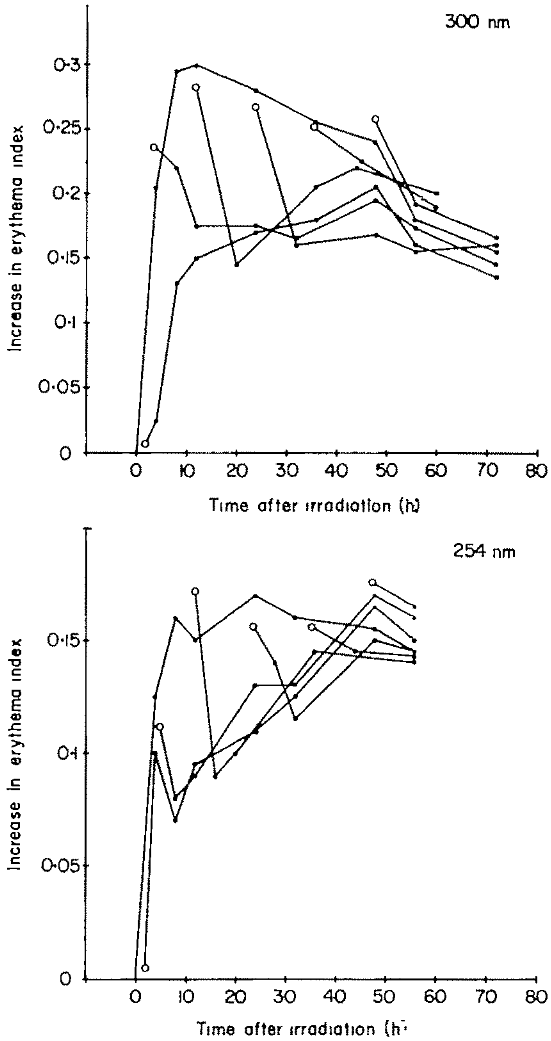

FIGURE 5. The effect of indomethacin, applied at different times after irradiation, on moderate erythema in one subject at each wavelength. The open circles indicate the time of application of indomethacin. The error bars have been omitted for clarity.

whom measurements were made at hourly intervals, significant inhibition first occurred 2 h after application for 300 nm erythema and 3 h after application for 254 nm erythema ($P < 0{\cdot}01$).

## DISCUSSION

We found that indomethacin caused inhibition of erythema due both to 300 and 254 nm radiation, but that the degree of inhibition was dependent both upon the time of observation after irradiation and the exposure dose of UVR. For moderate 254 and 300 nm erythema, at both

8 and 24 h after irradiation, the response to varying doses of indomethacin was similar, and this suggests that the mechanism of indomethacin inhibition was the same at these two wavelengths. Maximum suppression of both 300 and 254 nm erythema by indomethacin was achieved with a surface density of 100 $\mu g/cm^2$, and this dose was therefore used in all subsequent experiments.

Indomethacin applied immediately after irradiation caused inhibition within 4 h for 300 nm erythema, but with 254 nm erythema significant inhibition was not seen until 8 h after irradiation. However, when the skin was pre-treated with indomethacin 4 h before irradiation, significant inhibition of 254 nm erythema also occurred at 4 h. The subsequent time-course and intensity of erythema at both indomethacin and gel base pre-treated sites did not differ from those sites treated only after irradiation, suggesting that neither the gel base nor active gel had significant absorbent sunscreen action. This is in keeping with results from *in vitro* studies in which indomethacin, at a surface density of 100 $\mu g/cm^2$ (or equivalent volume of gel base) applied to isolated human epidermis, caused only minimal ($< 5\%$) change in transmission at wavelengths greater than 300 nm and approximately 20% reduction in transmission at 254 nm (unpublished data). As indomethacin was shown to act within 2–3 h of application, the observation that indomethacin inhibited 254 nm erythema at 4 h only when applied some time before irradiation suggests that, within the first few hours following irradiation, arachidonic acid release and subsequent metabolism by the cyclo-oxygenase pathway is greater at 254 nm than at 300 nm.

The time course of erythema inhibition after 4 h was similar for both 300 and 254 nm radiation and, 48 h after irradiation, indomethacin and gel base-treated sites were equally erythematous, irrespective of UVR exposure dose. Significant suppression of erythema occurred when indomethacin application was delayed until 24 h, indicating that prostaglandin synthesis remains increased until this time after irradiation. The degree of suppression, however, was no greater than that achieved by a single application immediately after irradiation, indicating that the eventual equal erythema of indomethacin and gel base-treated sites at 48 h is not explained by tachyphylaxis or inadequate dosage.

Eaglestein and Marsico (1975) found that indomethacin, given intradermally in a dose that caused a marked decrease in UVB erythema, had little or no effect on UVC erythema and suggested that different inflammatory mediators were responsible for erythema at these two wavelengths. The exposure dose of UVC was said to be equivalent to 3–5 MED (minimal erythemal dose) and the resulting erythema had faded to normal skin colour within 24 h. The UVB exposure dose was 5 MED, and the erythema persisted for more than 2 days. From the slope of the UVR-(log dose) erythema-response curves at these two wavelengths (Farr & Diffey, 1985), it is clear that the erythema resulting from 5 MED of UVC is much less intense (probably by a factor of 4) than that produced by 5 MED of UVB. As our results indicate that the magnitude of indomethacin inhibition is dependent upon the UVR dose (or degree of erythema achieved), the apparent difference in response of UVB and UVC erythema to indomethacin found by Eaglstein and Marsico (1975) would appear to be explained by the UVR doses that were employed. In keeping with this, Black *et al.* (1978b) reported that oral indomethacin caused partial inhibition of 6 MED UVC erythema.

Black *et al.* (1978b) found that the increase in prostaglandin concentration of suction blister fluid 24 h after irradiation with 3 MED of UVB was totally suppressed by oral indomethacin, but erythema was only inhibited partially [from 3 to 2 on a visual scale; mean output from a photo-electric plethysmograph reduced from 121 to 86 mV (29%)]. We found that topical indomethacin caused a mean reduction in $\Delta E$ from 0·175 to 0·102 (42%) when measured 24 h after a dose corresponding to approximately 3 MED at 300 nm. Although comparison of the results is difficult, it would appear that the degree of inhibition was similar, and that the dose of

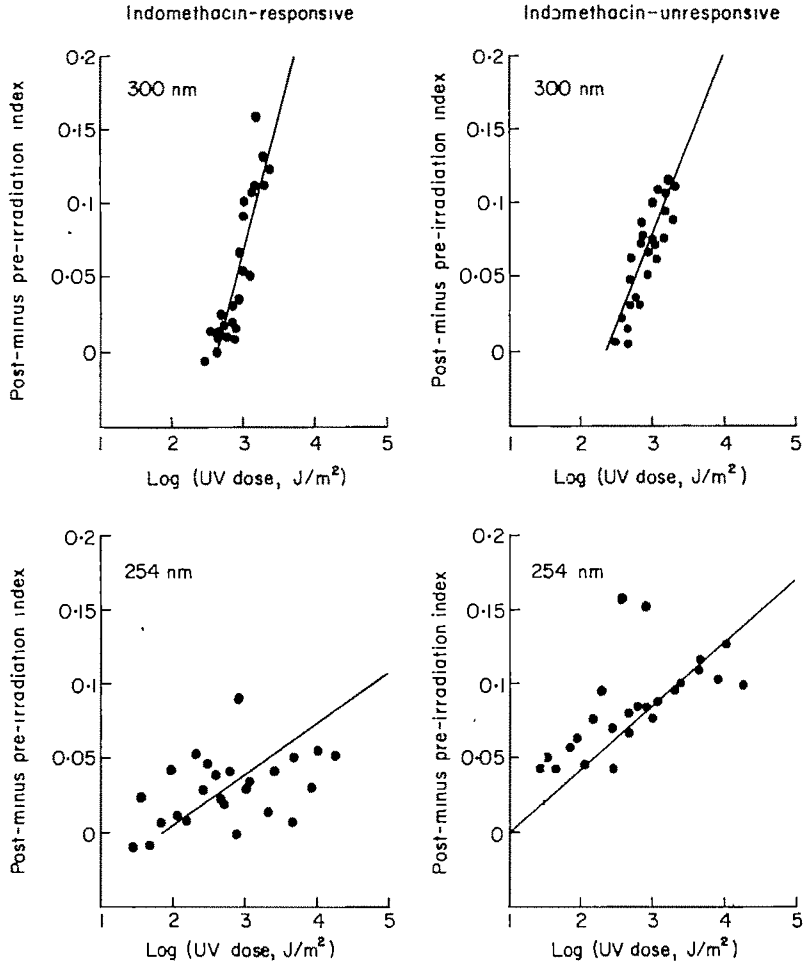

FIGURE 6. Pooled dose-response curves for the 'indomethacin-responsive' and 'indomethacin-unresponsive' components of 300 nm and 254 nm erythema 12 h after irradiation.

indomethacin that we used was also likely to achieve a similar degree of inhibition of prostaglandin synthesis.

Our data confirm findings (Black *et al.*, 1978b) that UVB and UVC erythema are inhibited only partially by indomethacin, and show that for both wavelengths the 'indomethacin-responsive' component occurs in the first 24 h after irradiation and is maximal at 4–8 h, and the 'indomethacin-unresponsive' component which, although present from 4 h, is also responsible for the later phase of erythema. The fact that 36 to 48 h after irradiation, both indomethacin and gel base-treated sites were equally erythematous suggests that these two components are independent. If the erythemal effectiveness of the two components is assumed to be linearly additive, the contribution of the indomethacin-responsive component to total erythema can be determined for any exposure dose of UVR by subtracting the increase in erythema index measured at the indomethacin treated site from that at the equivalent gel base-treated site.

Pooled dose-response curves for the indomethacin-responsive component at each wavelength 12 h after irradiation are illustrated in Figure 6. For each subject tested, at each log dose ($x$), the difference [$\Delta E_d(x)$] between the measured $\Delta E(x)$ at the gel base-treated site and the measured $\Delta E(x)$ at the corresponding indomethacin-treated site has been plotted using the transformation (Farr & Diffey, 1985):

$$\Delta E_d(\bar{t}-t+x) = \Delta E_d(x), \tag{1}$$

where $t$ is the threshold dose (defined as the intersect on the abscissa of the extrapolated linear portion of the dose-response curve for the gel base-treated sites in a given subject), and $\bar{t}$ is the geometric mean of the individual threshold doses at that wavelength. This transformation compensates for the difference in erythemal sensitivity between subjects but does not alter the calculated slope. Analysis of variance applied to regression (Armitage, 1971) was used to calculate the pooled slope, and this is represented by the straight line drawn through the data points. The relative contribution of the indomethacin-unresponsive component to total erythema is determined from the increase in erythema index at the indomethacin-treated sites. Pooled dose-response curves for the indomethacin-unresponsive component at each wavelength 12 h after irradiation are also shown in Figure 6. A similar transformation to Equation 1 has been used, and the pooled slope calculated as described above.

The pooled slope of the dose-response curves at 12 h for both components of erythema is considerably greater at 300 than at 254 nm. This shows that the difference in slope of the total erythema dose-response curve as these wavelengths is not explained by any difference in response to topical indomethacin and, therefore, that the difference in erythemal response to these two wavelengths is not due to the formation of different mediator substances. A three-dimensional representation of the two components of erythema is shown in Figure 7, where the increase in erythema due to each component is plotted according to both UVR dose and time after irradiation for a single subject at each wavelength. The slope of the dose-response curve for both components of erythema remains greater at 300 than at 254 nm, irrespective of time of observation after irradiation.

Black *et al.* (1980) showed that the concentration of $PGE_2$ in suction blister fluid following UVB irradiation was increased at 2 h, reached a maximum at 24 h and had fallen to control levels by 48 h. Following UVC irradiation, $PGE_2$ concentration reached a maximum at 18 h but was still elevated at 48 h (Camp *et al.*, 1978). It was suggested that the increased amount of $PGE_2$ at 48 h might explain the presence of UVC erythema at this time after irradiation. Our results show that for both wavelengths the indomethacin-responsive component of erythema is maximally effective between 4 and 12 h, and has minimal action after 36 h (Figs 5 and 7). The presence of erythema induced by UVR of either wavelength at 48 h is therefore not explained by the presence of cyclo-oxygenase metabolites. It is likely that the prostaglandin content of suction blister fluid represents activity of the cyclo-oxygenase pathway for a considerable period of time preceding the blister formation. Measurement of erythema during continued suppression by indomethacin would therefore seem to be a more accurate indicator of the time-course of the contribution of prostaglandins to UVR-induced erythema.

Reduction of delayed melanin pigmentation by indomethacin treatment of UVB erythema has been reported previously (Snyder & Eaglstein, 1974a; Snyder, 1975). Our results confirm this finding and show that UVC-induced delayed melanin pigmentation is also reduced by indomethacin. The mechanism of action of indomethacin on melanogenesis is not known. However, other epidermal responses to UVR, such as keratinocyte cell death and altered DNA synthesis, do not appear to be attenuated by indomethacin (Snyder, 1975).

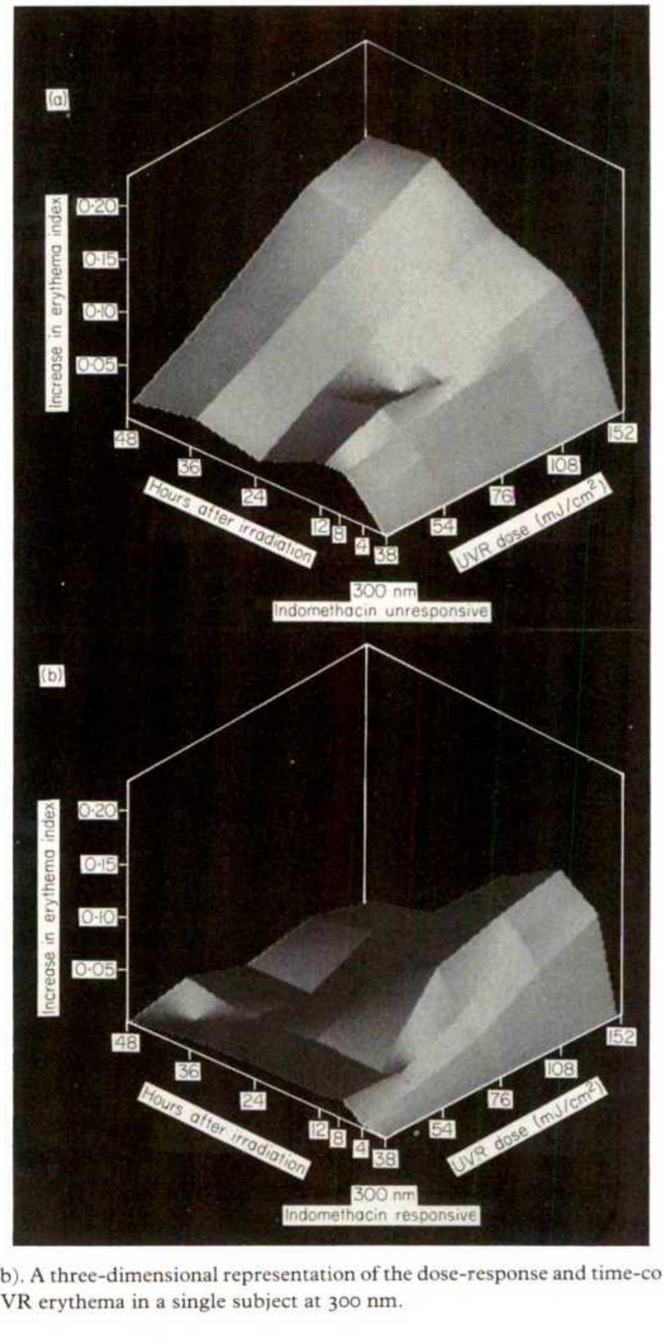

FIGURE 7(a) and (b). A three-dimensional representation of the dose-response and time-course of the two components of UVR erythema in a single subject at 300 nm.

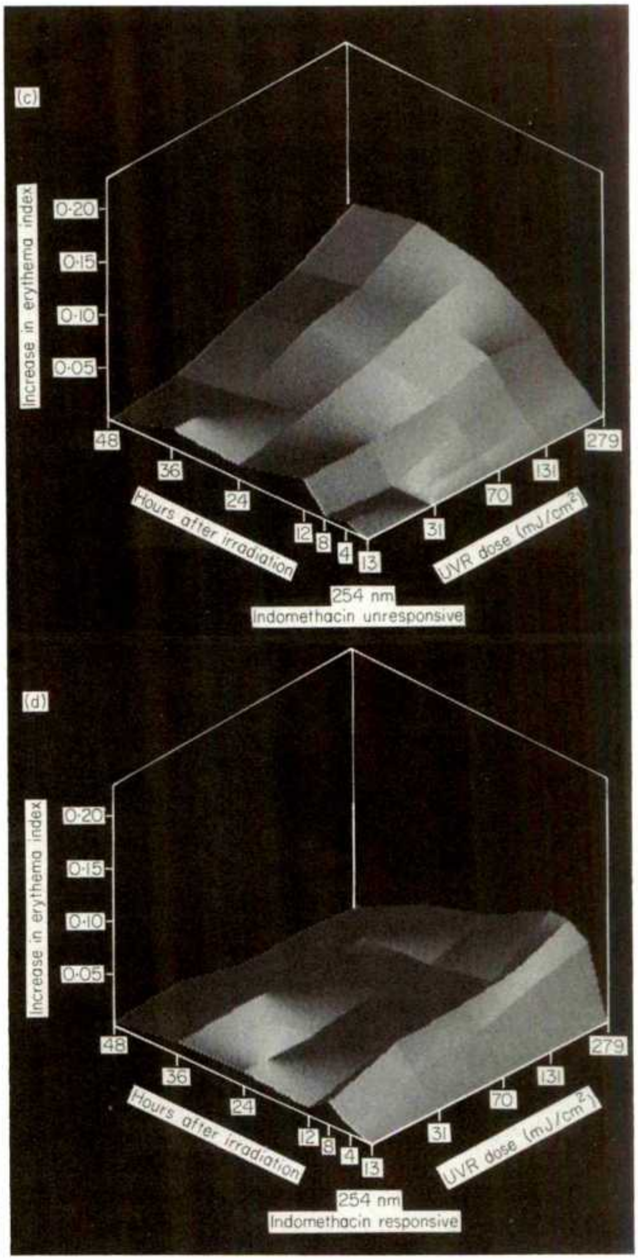

FIGURE 7(c) and (d). A three-dimensional representation of the dose-response and time-course of the two components of UVR erythema in a single subject at 254 nm.

Observations on the time to appearance and disappearance of the erythema induced by both 254 nm (UVC) and 300 nm (UVB) radiation are in accordance with a diffusion theory model (van der Leun, 1966) in which mediator substances, formed in the epidermis, diffuse to the dermis and cause vasodilation (Farr & Diffey, 1985). The present study has shown that the difference in slope of the UVR-(log dose) erythema-response curve for 300 and 254 nm radiation is not explained by the formation of different mediator substances. Other factors, such as the mechanisms of mediator release, quantum yield of the radiation, or the site of action of the mediators on the dermal vasculature may be important.

## ACKNOWLEDGMENT

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# Behavioural treatment of scratching in patients with atopic dermatitis

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## SUMMARY

A behavioural method of habit reversal, in combination with a hydrocortisone cream, was compared with the use of cream alone in the treatment of 17 patients with atopic dermatitis. The patients were assigned randomly to two groups, one of which received the combination treatment and the other regular ointment treatment. The patients' skin status was assessed before and after treatment, and the patients recorded their scratching during the study. Both groups improved, but the group which received habit-reversal therapy improved significantly more. A strong correlation was found between reduction in scratching and improvement in skin status.

Atopic dermatitis is a skin disorder characterized by a dry, irritable and itchy skin associated with and exacerbated by excessive scratching. The skin lesions are commonly seen on the flexures of the arms and legs, and on the nape of the neck, which are areas easily accessible to scratching. The skin condition often begins or worsens in winter when the humidity of the air is low.

The role of scratching in perpetrating skin disorders is not fully understood; it is not known whether the histological and histochemical changes in atopic dermatitis are the results of scratching or are directly related to some somatic disturbance.

A study by Golblum and Piper (1954) on patients with lichen simplex suggests that scratching causes some of these changes, as they were able to induce patches of lichenification on normal areas of the skin by the use of a scratching machine. Another study by Scott (1962), on atopic patients, showed that the histochemical changes probably occur after the lesions have developed.

Most skin disorders involving dry skin produce itching even if they are not irritated by scratching. The role of scratching then is to decrease immediate itching, but may lead to further itching by aggravation of the skin disorder. This immediate reduction of itching serves as a strong reinforcer for scratching behaviour, and this increases the probability of subsequent

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scratching in response to itching (Bär & Kyupers, 1973). Scratching as a conditioned response has been investigated in a study by Robertson, Jordan and Whitlock (1975) who were able to show that the scratch response in patients with lichen simplex was more readily conditioned and more slowly extinguished than in control subjects. The most obvious interpretation of these findings is that scratching in people with certain skin disorders was much more reinforced than in normals, either because of a stronger itch reducing effect, or because of a long history of scratching.

Several approaches have been suggested for treating the scratching associated with dermatological problems—the majority with a behavioural perspective. All studies have been done on more or less controlled single cases, and the rationales behind the various methods used are rarely given. However, most studies have included reinforcement of the development of non-scratching behaviours (Allen & Harris, 1966; Bär & Kyupers, 1973; Cataldo *et al.* 1980; Dobes, 1977; Ratliff & Stein, 1968; Rosenbaum & Ayllon, 1981; Watson, Tharp & Krisberg, 1972). With the exception of the study by Watson *et al.* (1972), all studies attempted to make the patient ignore the itching, thereby causing it to decrease gradually as the skin healed.

As itching produces such a strong urge to scratch, it seems logical, at least in the initial phase, to use a treatment method where the itching is reduced without causing damage. A comprehensive behavioural treatment programme should combine the striking and patting method described by Watson *et al.* (1972) with the powerful habit reversal method originally developed by Azrin and Nunn (1973), and later used successfully on scratching by Rosenbaum and Ayllon (1981).

The study by Cataldo *et al.* (1980) is one of the few which reported the progress of their patient's skin condition. Continuous assessment of the patient's skin condition is very important as such progress cannot be taken for granted. It is still unclear whether a patient's healing rate is faster if he or she gives up scratching.

The present study was designed to test the effectiveness of modified habit-reversal treatment in patients with atopic dermatitis receiving corticosteroid treatment. Both skin status and scratching were monitored to determine whether there was a correlation between the two.

## METHODS

### *Subjects*

Seventeen patients with a diagnosis of atopic dermatitis, aged 19–41 years (mean 30·5 years), were recruited from a dermatology clinic. Criteria for acceptance into the study were (a) age between 18 and 45 years, (b) dermatitis present for at least 3 years immediately prior to the study, (c) consultation with at least one physician about the dermatitis during that period, and (d) no obvious psychiatric problems.

### *Procedure*

Initially, all patients were seen by a dermatologist who assessed their dermatitis, explained the aim of the study and obtained their informed consent. The patients were given instructions about the information which they were to record daily during the study, beginning with a 7 day pre-treatment period. The patients were randomized into two groups. One group was treated with corticosteroid ointment plus a behavioural habit-breaking method (M + B), and the other group with corticosteroid ointment only (M). One patient dropped out during the pre-treatment period. Before entering the trial, the patients completed a credibility questionnaire

(Borcovec & Nau, 1972) to control for initial differences in expectations between the two groups. The results showed no difference between the two groups.

After a 7 day baseline period without treatment, both groups started treatment with a new cream containing natural moisturizing factors in combination with hydrocortisone (Sential®, Cortesal®). Each patient in the M+B group received additionally two sessions of the psychological habit-breaking treatment during the first week of the treatment period.

After 28 days of treatment, the patients were again assessed by the dermatologist.

### *Measurements*

*Medical assessment.* During the pre- and post-treatment assessments each patient's skin was graded for dryness, erythema, infiltration and scaling on a 0–3 scale (0 = no symptoms, 1 = mild, 2 = moderate, and 3 = pronounced symptoms).

*Annoyance questionnaire.* Before and after treatment the patients completed a questionnaire about scratching, itching, their annoyance with treatment restrictions, and cosmetic problems.

*Scratching.* The patients were provided with a golf counter to monitor scratching. It was to be used each day during waking hours, to record when the patient scratched. The counter was to be pressed once for each episode (one episode could contain several scratches). Each evening the day's total was tranferred to a form, which was sent to the doctor at the end of each week.

*Itching and scratching in 'worst situation'.* Each patient was asked to identify the situation in which he or she felt the strongest urge to scratch, and was then provided with a form on which to record an assessment of the urge to scratch and scratching episodes during a fixed interval in that situation. The patient was asked to fill in the number of scratching episodes, the intensity of the urge to scratch (rated 0–6), and the localization of the urge (local or global).

*Compliance with the ointment regimen.* To estimate their compliance with the ointment regimen, patients were instructed to record on the scratching form each day whether they had applied the cream as instructed.

### *Treatment*

*Medical treatment.* Both groups of patients were given identical detailed instructions on applying the hydrocortisone cream to their eczema.

*Behavioural treatment.* The psychological treatment was given only to one of the groups (M+B) and was based on a behavioural technique originally developed by Azrin and Nunn (1973, 1977), and later modified by Rosenbaum and Ayllon (1981), for patients with neurodermatitis. Initially, the patients described and demonstrated their scratching in the 'response description' procedure. The next step was to teach the patients to recognize when they were bringing their hands towards the areas which they scratched ('early warning'). The patients were then taught 'situation awareness', which consisted of the patient describing those situations in which they usually scratched or did so more actively. To increase the patient's motivation for treatment, unpleasant aspects of scratching were discussed ('habit control motivation').

In the 'competing response practice' procedure the patients were taught to develop behaviours incompatible with scratching. As itching produces a strong urge to scratch, it was considered necessary to practice the competing response in two steps. In the first step the patients were instructed to put their hands firmly on the itching area and keep them there for 1 min before moving them to the side of their thighs or to grasp some other object. In the second step, which was practised during the second session, the patients were taught to move their hands directly to their thighs, or to grasp an object. To train generalization into out of therapy situations, the patients were asked to choose situations in which scratching usually occurred, and practise the competing response while pretending to be in those situations ('symbolic rehearsal'). In the last part of the session, the patients were engaged in a discussion during which they had been instructed to use the competing responses whenever they felt an urge to scratch or actually scratched. If they failed to do so they were reminded by the therapist.

### *Statistical analysis*

Students unpaired *t*-test was used for between-group comparisons, and paired *t*-tests for comparing pre- and post-treatment within groups.

## RESULTS

### *Medical assessment*

Figure 1 shows the reduction in skin irritation scores in the two groups. The mean reduction in total score for the M + B group was 67% and for the M group 37%. Skin irritation scores were reduced significantly in both groups but the total score reduction in the M + B group was significantly greater than in the M group ($P < 0{\cdot}05$).

As can be seen in Figure 1, the M + B group improved in all four measures of irritation, while the M group improved in only two. A significant difference between the two groups was found in infiltration ($P < 0{\cdot}05$) and in erythema ($P < 0{\cdot}05$).

### *Annoyance questionnaire*

The results of the annoyance questionnaire are given in Figure 2. There was a significant

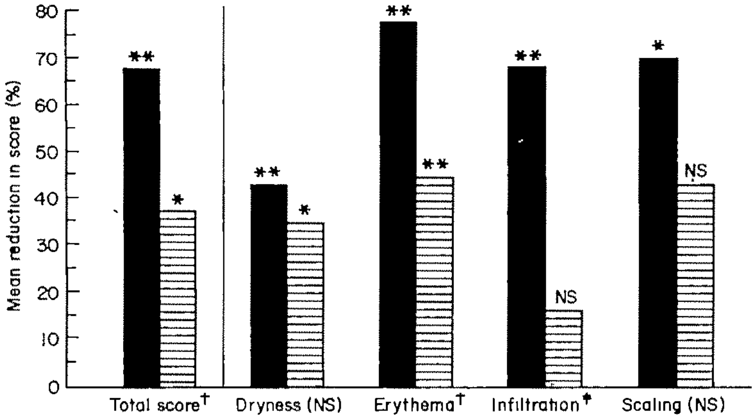

FIGURE 1. Changes in severity of eczema symptoms in seven patients treated with ointment and behavioural therapy (M + B) (■), and in nine patients treated with ointment only (M) (▤). Comparison with pre-treatment scores $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$. Comparison between the two groups $^{\dagger}P < 0{\cdot}05$; $^{\ddagger}P < 0{\cdot}01$. NS = not significant.

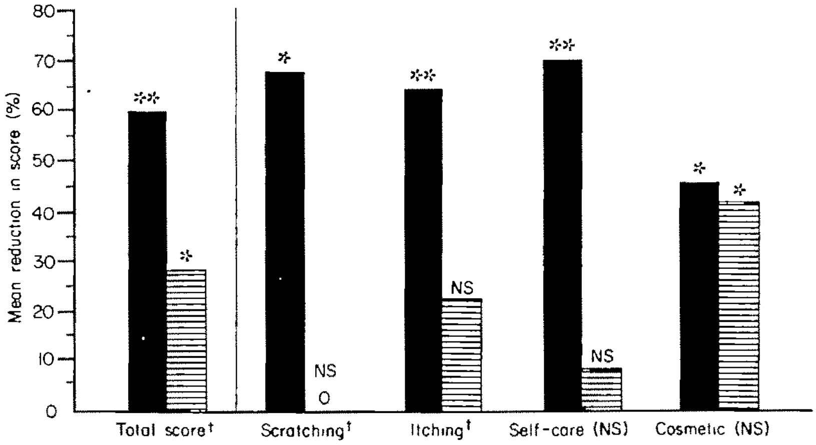

FIGURE 2. Changes in self-assessed annoyance in seven patients treated with ointment and behavioural therapy (M+B) (■), and in nine patients treated with ointment only (M) (▤). Comparison with pre-treatment scores $^{*}P<0{\cdot}05$; $^{**}P<0{\cdot}01$. Comparison between the two groups $^{\dagger}P<0{\cdot}05$. NS = not significant.

reduction in total annoyance scores in both groups; a reduction of 61% in the M+B group ($P<0{\cdot}01$), and 27% in the M group ($P<0{\cdot}05$). The difference in annoyance scores between the groups was also significant ($P<0{\cdot}05$).

The M+B group showed a significant reduction in all four annoyance scores, scratching, itching, self-care and cosmetic problems ($P<0{\cdot}05$), while the M group only showed a significant reduction in cosmetic problems. There were significant differences in annoyance between the groups for scratching ($P<0{\cdot}05$) and for itching ($P<0{\cdot}05$), i.e. the two areas where scratching is of immediate importance. The M+B also showed a greater reduction in annoyance with self-care than the M group, but this did not reach statistical significance ($P<0{\cdot}08$).

### *Scratching*

The scratching frequencies before and after treatment are shown in Table 1. Both groups showed a significant reduction in scratching ($P<0{\cdot}05$), but the M+B group showed a significantly greater reduction than the M group ($P<0{\cdot}01$).

TABLE 1. The daily frequency of scratching

| | | Number of episodes/day | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Pre-treatment | | Post-treatment | | Improvement | | |
| Group | No. of patients | mean | (range) | mean | (range) | mean | % | $P$ |
| M+B | 7 | 92 | (8–220) | 24 | (0·5–113) | 68 | 79 | $<0{\cdot}05$ |
| M | 9 | 90 | (15–202) | 45 | (6–95) | 44* | 49 | $<0{\cdot}01$ |

* Significantly greater improvement in M+B group than in M group: $P<0{\cdot}01$.

TABLE 2. The frequency of scratching in 'worst situation'

| Group | No. of patients | Number of episodes/day | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Pre-treatment | | Post-treatment | | Improvement | | |
| | | mean | (range) | mean | (range) | mean | % | $P$ |
| M+B | 6 | 10 | (4–25) | 1 | (0–4) | 8 | 83 | $<0.05$ |
| M | 6 | 12 | (6–19) | 5 | (0·1–10) | 7* | 63 | $<0.01$ |

* There was no significant difference between the improvements in the two groups.

*Itching and scratching in 'worst situation'*

This measure was rather difficult for the patients to record and, because of that, data on scratching are missing for four patients and on itching for six patients. Thus, the results must be interpreted with caution.

Both groups reduced significantly their scratching in their 'worst situation' (Table 2). No statistically significant difference between the two groups could be shown. There was no significant change in itching in either group (Table 3).

*Compliance with ointment regimen*

The average number of days/week the patients applied the ointment in the M+B group was 6 (range 5–7) and in the M group 5 (range 2–7). There was no statistically significant difference between the groups.

*Correlations between improvement in skin status, scratching and itching*

A Pearson product-moment correlation was calculated for both groups between percent improvement in skin status, assessed by the dermatologist, and percent reduction in frequency of scratching, assessed by the patient: $r=0{\cdot}74$ ($P<0{\cdot}001$). The correlation between percent

TABLE 3. The intensity of itching in 'worst situation'

| Group | No. of patients | Score | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Pre-treatment | | Post-treatment | | Improvement | | |
| | | mean | (range) | mean | (range) | mean | % | $P$ |
| M+B | 4 | 3 | (2–4) | 2 | (0·8–4) | 1 | 37 | NS |
| M | 6 | 3 | (2–4) | 2 | (0·3–4) | 1* | 27 | NS |

* There was no significant difference between the two groups.

improvement in scratching and improvement in skin status and improvement in itching was $r=0{\cdot}51$ ($P<0{\cdot}05$).

## DISCUSSION

The results of the present study show that a behavioural treatment, superimposed on a regular corticosteroid ointment regimen, can improve considerably the skin status of patients with atopic dermatitis. The mechanism behind this relative improvement is a reduction in the patients' frequency of scratching. This is in accordance with results reported by Rosenbaum and Ayllon (1981) on frequency of daily scratching. A problem with the comparison of the two studies is that due to differences in definition of scratching, the initial frequency of scratching differs considerably. In Rosenbaum and Ayllon's study (initial frequency 30 scratches/day), scratching was to go on for 5 s to be counted while, in the present study (initial frequency 92 scratches/day), all scratching episodes were registered. However, if the percentage reduction of scratching in the two studies is compared, the results are similar (79% compared with 75%).

This is probably the first experimental group study showing that a reduction in scratching produced by a behavioural treatment method is of importance in improving eczema. However, our design did not control for all alternative interpretations of the results. One is that the two sessions of extra interest and care that the patients in the M + B group received were sufficient to produce the effect. However, some studies (Allen & Harris, 1966; Walton, 1960; Carr & McDowell, 1980) have shown that attention may be a reinforcer maintaining scratching behaviour. Thus, the extra attention the M + B group received would rather be expected to be counterproductive to a favourable outcome.

The highly significant correlation between skin status and scratching means that 55% of the improvement in skin status could be accounted for by the reduction in scratching. Therefore, we strongly recommend that any treatment programme for atopic eczema should include steps to reduce scratching.

The itching results are somewhat equivocal as there was no reduction in itching in 'worst situation', but a considerable reduction on the annoyance questionnaire. Furthermore, that reduction was strongly correlated with reduction in scratching and somewhat less strongly correlated with improvement in skin status. Although it is reasonable to assume that itching, for most patients, was the most important negative reinforcer to initiate and maintain scratching, the exact role of itching was not investigated in this study. Questions remain as to whether the immediate urge to scratch increases or decreases when the patient abstains from scratching, what psychological factors or external stimuli trigger itching, and whether itching is always localized to the patient's eczematous areas, or whether there is a global itching urge.

The study did not address itself to what happens after longer periods of time. If conditioning factors are involved and have not been taken into account, the patient is still at risk of having a relapse into excessive scratching even after the eczema is healed. Apart from longer follow-up periods, the solution to this problem would seem to require individual behaviour analyses and programmes.

## ACKNOWLEDGMENT

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# Effect of percutaneous absorption of hydrocortisone on adrenocortical responsiveness in infants with severe skin disease

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## SUMMARY

Percutaneous absorption of hydrocortisone was studied in 18 children (aged from 6 weeks to 14½ years) with atopic or seborrhoeic dermatitis, by measuring their serum cortisol before and after application of 1% hydrocortisone cream. Endogenous secretion of cortisol was suppressed with dexamethasone. A 24 h absorption test was performed on nine children. In six, percutaneous absorption was detected. The highest serum cortisol level was reached within the first 6 h. A 4 h absorption test was developed on the basis of the 24 h test. This short absorption test was performed on nine children, and in eight of them absorption of hydrocortisone was detected. The rise of serum cortisol ranged from 98 to 2669 nmol/l. The 2 h ACTH test was performed to evaluate the effect of previous treatment with topical glucocorticoids. Suppressed adrenocortical function was found in five of 13 children, and was associated significantly with high post-application serum cortisol levels. This occured more often in infants with a severe skin disorder than in older children or in those with mild or moderate skin disease.

The percutaneous absorption of glucocorticoids has been reported previously (Gemzell, Hård & Nilzen, 1955; Feinblatt *et al.*, 1966; Feiwel, 1969; Munro, 1976; Weston *et al.*, 1980; Bode, 1980; Hehir *et al.*, 1983; Bartorelli & Rimondini, 1984). Systemic side-effects, such as adrenal insufficiency, growth retardation, Cushing's syndrome, arterial hypertension, and intracranial hypertension on withdrawal of topical treatment, have been associated mainly with the use of halogenated glucocorticoids (Freiwel, 1969; Keipert & Kelly, 1971; Munro, 1976; Weston *et al.*, 1980; Bode, 1980; Bartorelli & Rimondini, 1984). Therefore, hydrocortisone, instead of the more potent glucocorticoids, is recommended for children, although systemic side-effects during topical treatment with hydrocortisone have also been reported (Fanconi, 1962; Feinblatt

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*et al.*, 1966; Feiwel, 1969; Hosking & Elliston, 1978). The purpose of the present study was to measure the percutaneous absorption of hydrocortisone by a new direct method. Adrenal function was tested in order to evaluate the effect of previous long-term treatment. A child with Cushing's syndrome due to percutaneous absorption of glucocorticoids is also presented.

## METHODS

### *Patients*

Eighteen children (aged 6 weeks to 14½ years), all except one in good general health, with chronic skin disorders of varying severity were selected for the study. One patient (Patient 4) had a clearcut Cushing's syndrome due to topical steroid treatment of his seborrhoeic dermatitis. All the children had been admitted to hospital due to exacerbation of their skin disorder. The extent of skin involvement was determined using a burn assessment chart (Vaughan, McKay & Behrman, 1979), and varied from 30 to 90% (mean 71%). The state of the skin was scored by grading the erythema and excoriations, 0 – none, 1 – mild, 2 – moderate, and 3 – severe. The clinical details of the patients are presented in Table 1.

### *Serum cortisol determination*

Serum cortisol levels were determined using the Cortisol ($^{125}$I) radioimmunoassay kit manufactured by Farmos Diagnostica, Turku, Finland. In this test the cross reactivity with dexamethasone is less than 0·005%.

### *The 24 h absorption test*

A 24 h absorption test was performed on nine children during the first few days after admission to hospital. Topical hydrocortisone was stopped for 36 h prior to the test. The serum samples for cortisol determination were taken through an intravenous plastic cannula in the morning before, and 1, 2, 3, 4, 5, 6, 8, 12, 18 and 24 h after, the application of 1% hydrocortisone cream. The cream did not contain propylene glycol. No occlusion was used. Endogenous secretion of cortisol was suppressed with dexamethasone in all the children, except Patient 4, who already had severe adrenal insufficiency. The dose of dexamethasone was 0·25 mg if the body surface was under 0·5 m$^2$, 0·5 mg between 0·5–0·99 m$^2$, 0·75 mg between 1·0–1·49 m$^2$ and 1·0 mg for body surface over 1·5 m$^2$. This dose was given 3 times a day to Patients 1, 3, 5, 7 and 8. Because of ineffective suppression in these patients, the rest of the patients received dexamethasone four times a day. Dexamethasone was given at 7 pm, 1 am, and 7 am before the first blood sample, which was taken at 8 am. Thereafter, dexamethasone was given every sixth hour until the last sample had been taken. This dose schedule gave good suppression of endogenous cortisol secretion as evidenced by the subnormal basal levels of serum cortisol in these patients with a single exception (Patient 11). This can also be seen in the 24 h suppression curve of Patient 10 without application of hydrocortisone (Fig. 1b). To evaluate the effect of stress due to insertion of the intravenous cannula, serum cortisol was measured at the time of the procedure and 1 and 2 h thereafter, without application of hydrocortisone in four patients who had received the dexamethasone suppression. In three of these patients no rise of serum cortisol was observed, and in one the serum cortisol rose from 46 to 107 nmol/l, which was still clearly below the suppressed level (200 nmol/l).

### *The 4 h absorption test*

A 4 h absorption test was developed on the basis of 24 h test and performed on nine children. In

TABLE 1. Clinical details of patients

| Patient no. | Age in months (m) or years (y) | Sex | Diagnosis | Body surface (m²) | % covered by dermatitis | Amount of cream used in the tests (g) | Duration of hydrocortisone use prior to the study | Scores for | | Adrenocortical function suppressed (ACTH test) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Erythema | Excoriations | |
| 1 | 3 m | M | AD | 0·32 | 70 | 11·9 | 0 | 1 | 1 | ND |
| 2 | 5 m | M | AD | 0·33 | 80 | 13·1 | 3 months | 1 | 2 | + |
| 3 | 6 m | M | SD | 0·28 | 70 | 11·0 | 2 months | 0 | 1 | — |
| 4 | 14 m | M | SD | 0·33 | 40 | 10·2 | 14 months* | 3 | 2 | + |
| 5 | 3 y 9 m | M | Ich | 0·43 | 60 | 36·4 | years* | 0 | 2 | + |
| 6 | 6 y 2 m | F | AD | 0·80 | 70 | 29·1 | years | 0 | 1 | — |
| 7 | 6 y 8 m | F | AD | 0·92 | 80 | 58·6 | years | 0 | 1 | — |
| 8 | 8 y 7 m | F | AD | 0·94 | 80 | 54·6 | years | 0 | 1 | — |
| 9 | 14 y 5 m | M | AD | 1·72 | 90 | 106·0 | years | 1 | 2 | — |
| 10 | 8 y 8 m | F | AD | 1·05 | 90 | 0 | years | 1 | 2 | ND |
| 11 | 1 5 m | F | SD | 0·27 | 90 | 15·4 | 0 | 1 | 2 | ND |
| 12 | 6 m | F | SD | 0·33 | 40 | 13·0 | 1 month | 1 | 1 | ND |
| 13 | 7 m | F | AD | 0·42 | 30 | 4·5 | 2 months | 1 | 1 | — |
| 14 | 8 m | M | AD | 0·36 | 80 | 17·9 | 2 months | 1 | 1 | — |
| 15 | 12 m | M | AD | 0·42 | 90 | 10·1 | 5 months | 3 | 1 | + |
| 16 | 2 y 3 m | F | AD | 0·52 | 90 | 38·2 | 3 months | 0 | 2 | ND |
| 17 | 4 y 1 m | F | AD | 0·64 | 50 | 11·4 | 4 months | 0 | 1 | — |
| 18 | 8 y 8 m | F | AD | 1·05 | 90 | 41·8 | years | 0 | 1 | ND |
| 19 | 11 y 8 m | M | AD | 1·27 | 80 | 55·4 | years* | 1 | 3 | + |

AD, Atopic dermatitis.
SD, Seborrhoeic dermatitis.
Ich., Ichthyosis.
* More potent glucocorticoids also used briefly
+, Suppressed; —, normal.
ND, not tested.

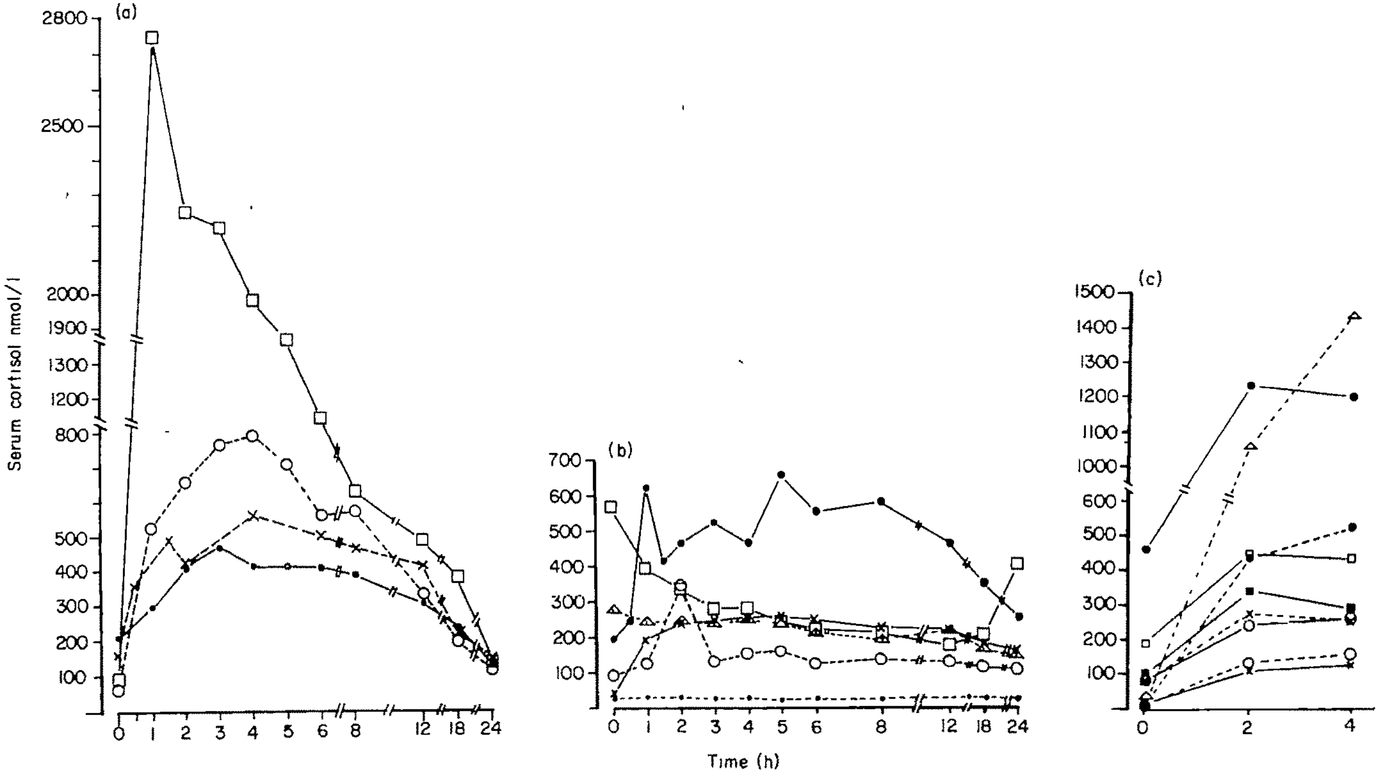

FIGURE 1. Individual hydrocortisone absorption curves. Serum cortisol levels measured before (time 0) and after application of 1% hydrocortisone cream. (a): 24 h test. Patients: 1, ●; 2, ○; 3, ×; and 4, □. (b): 24 h test. Patients: 5, ●; 6, □; 7, ○; 8, △; 9, ×; and 10, - -●- -. (c): 4 h test. Patients: 11, ●; 12, ○; 13, - -×- -; 14, ■; 15, △; 16, □; 17, ×, 18, - -○- -; and 19 - -●- -.

this short absorption test, serum cortisol samples were taken before, and 2 and 4 h after, the application of the cream, but otherwise the test was performed in the same way as the 24 h test. In the 4 h test the three samples were taken through an intravenous cannula, or separately through a skin area without hydrocortisone application.

### *The 2 h ACTH test*

Synthetic tetracosactide (S-Cortophin, N.V. Organon, Oss, The Netherlands) was given as an i.v. bolus injection of 0·25 mg/1·73 $m^2$ at 8–9 am. Blood samples were taken before and 1 and 2 h after the injection (Leisti & Perheentupa, 1978). The ACTH test was performed on 13 children either before or one week after the absorption test, when the dexamethasone suppression would no longer be occurring (Liddle, 1960).

### *Statistics*

Statistical comparison of the group means was performed using Student's *t*-test.

## RESULTS

### *Absorption tests*

Patients 1, 6 and 11 had basal suppressed cortisol levels above the suppression level (>200 nmol/l). Because of ineffective suppression, the absorption of hydrocortisone cannot be definitely evaluated in these patients. The individual absorption curves are presented in Figure 1. The 24 h absorption test was performed on nine children (Patients 1–9; Fig. 1a and b). In six of these, percutaneous absorption of hydrocortisone caused a rise in serum cortisol ranging from 212 to 2669 nmol/l. The highest value was reached within the first 6 h (mean 3·2 h).

The 4 h absorption test (Fig. 1c) was performed on nine children, and in eight of these absorption of hydrocortisone caused a rise in serum cortisol ranging from 98 to 1405 nmol/l.

### *The 2 h ACTH test*

The 2 h ACTH test was performed on 13 children. The results are presented in Figure 2. The serum cortisol responses to tetracosactide were subnormal in five children. The mean rise in serum cortisol in the absorption tests was significantly higher in the five children with suppressed adrenocortical function (1157 ± 925 nmol/l) than in the six children with normal adrenocortical function (251 ± 99 nmol/l) ($P < 0·05$). Patients 6 and 8 had a normal response, but they were excluded from the calculation because the percutaneous absorption could not be evaluated due to ineffective suppression.

## CASE REPORT

Patient 4, a 22-month-old boy with severe seborrhoeic dermatitis, had been treated with glucocorticoid creams daily since birth. The creams contained mostly 1% hydrocortisone. At the age of 9 months he was also treated briefly with halogenated glucocorticoids. Histiocytosis or congenital graft-versus-host reaction were suspected on the basis of the severe dermatitis, recurrent bacterial infections (osteomyelitis, sepsis and skin infections), growth retardation and hepatomegaly. However, these diagnoses could not be verified in spite of extensive investigations, including skin, lymph node, bone marrow and open liver biopsies. Features of Cushing's syndrome (Fig. 3), growth retardation since the age of one month (Fig. 4) and adrenal insufficiency (basal level of serum cortisol 81 nmol/l) were noted at the age of one year. The 24 h

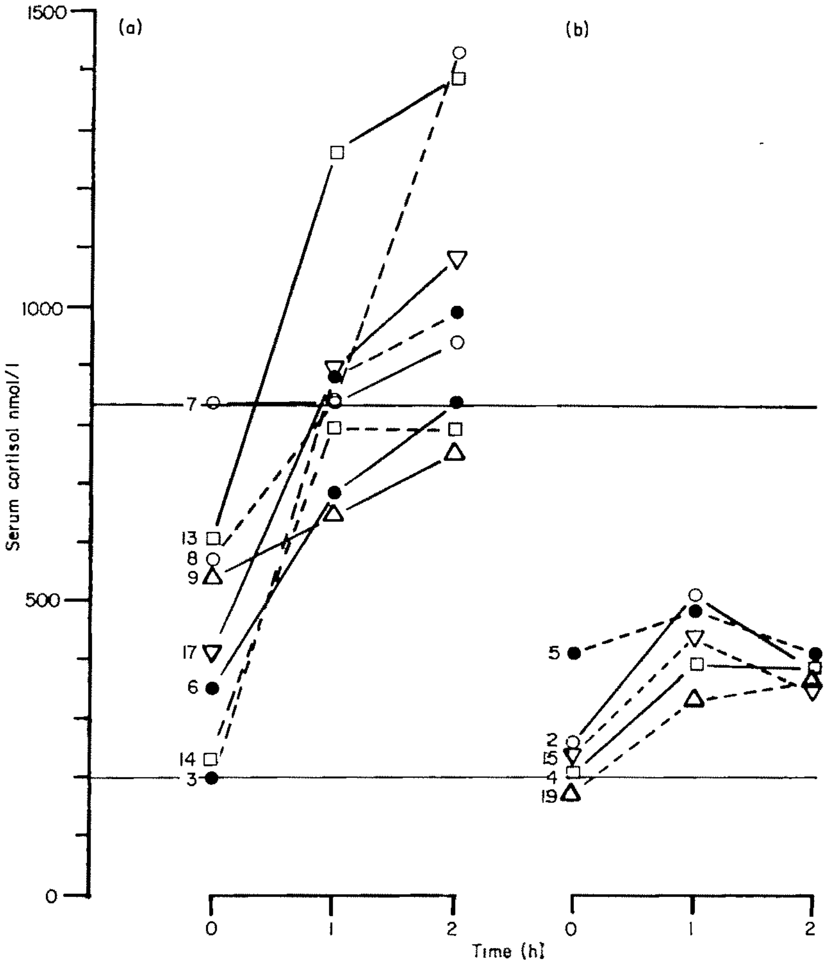

FIGURE 2. The individual results of the 2 h ACTH test. Horizontal lines indicate the lower limit of the normal morning basal serum cortisol level (200 nmol/l) and the lower limit of the normal response (2 h serum cortisol 830 nmol/l). (a) Eight children with normal or near normal response; (b) five children with adrenocortical suppression. Numbers are patient numbers.

absorption test revealed massive percutaneous absorption of hydrocortisone with a rise in serum cortisol to 2669 nmol/l (Fig. 1a). Because of recurrent skin infections, sulphafurazol and trimethoprim were given on alternate weeks. Hydrocortisone concentration was gradually diminished in creams from 1 to 0·5% and finally to 0·25% over 3 months. In an attempt to mimic the physiological secretion of cortisol, applications were made mainly in the morning. The state of the dermatitis did not deteriorate in spite of the diminished use of topical hydrocortisone. After 10 weeks, a normal morning level of serum cortisol (208 nmol/l) was reached, but the response to ACTH was still subnormal (2 h serum cortisol concentration 384 nmol/l). Five and a half months after the beginning of diminished use of hydrocortisone, the ACTH test showed nearly normal adrenal function (2 h serum cortisol concentration 606 nmol/l). The catch-up

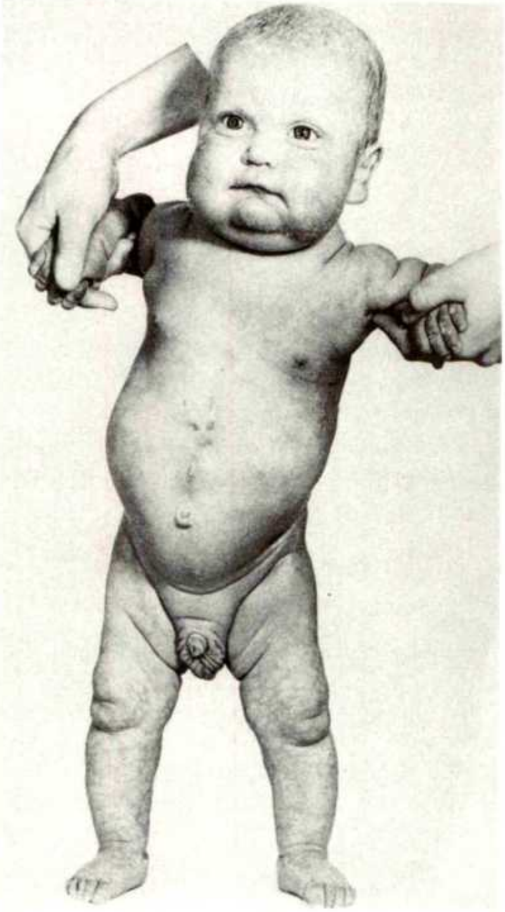

FIGURE 3. The patient with Cushing's syndrome (Patient 4), at the age of 1 year.

growth still continued at the age of 22 months, and at that time a normal response to ACTH was reached (2 h serum cortisol 980 nmol/l). The dermatitis was still well controlled with 0·25% hydrocortisone cream and continuous sulphafurazol and trimethoprim therapy.

## DISCUSSION

The total serum cortisol measured during the absorption test contained both endogenous and absorbed cortisol. Dexamethasone used in this study to suppress the endogenous cortisol is not measured by the determination of serum cortisol. The suppressive effect of dexamethasone is seen in the subnormal basal levels of serum cortisol in the majority of the children, and also in the low serum cortisol concentrations in the control patient, who did not receive any hydrocortisone. The absorption test was performed in the morning, when the serum cortisol is

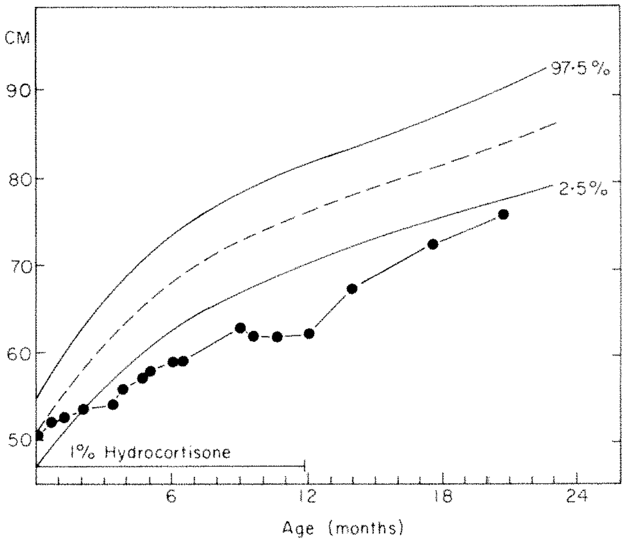

FIGURE 4. The growth curve of Patient 4.

normally at its highest. Stress may cause a rise in serum cortisol. The pain due to procedure is regarded as a Class I stimulus, which does not cause a release of ACTH under dexamethasone suppression (Yates, 1969). In this study the insertion of the intravenous cannula did not cause a rise in serum cortisol. These facts confirm that the rise in serum cortisol after the application of hydrocortisone consists of absorbed hydrocortisone.

The measurement of absorption is reliable only if the suppression is successful (supressed cortisol level < 200 nmol/l). After the application, falsely high cortisol levels may be detected if the samples are taken through skin areas on which hydrocortisone has been applied. This is best avoided by using an intravenous cannula instead of taking samples separately.

The area of the graph between the absorption curve and the basal cortisol level describes the absorption better than does the maximum concentration of serum cortisol (Patient 7 compared with Patient 9 in Fig. 1b). It is notable that the shapes of the absorption curves of hydrocortisone resemble closely those of clobetasol propionate (Hehir *et al.*, 1983). In that study, performed in adults, the peak value of absorption was also reached within the first 6 h both in patients with psoriasis and in patients with eczema.

The percutaneous absorption of hydrocortisone seems to be higher in infants than in older children (Fig. 1a compared with 1b). However, most studies of the barrier function of the skin have not shown any significant difference in percutaneous absorption between infants and adults (Wildnauer & Kennedy, 1970; Nachman & Esterly, 1971; Cunico *et al.*, 1977; Harpin & Rutter, 1983). The high post-application cortisol levels may be explained by the greater ratio of surface area to weight in infants (Wester *et al.*, 1977). Whatever the explanation, infants with severe skin disorders seem to have a greater risk of systemic side-effects than do older children, due to higher post-application serum cortisol levels. In the present study, high post-application serum cortisol levels were associated significantly with suppressed adrenocortical function.

A high degree of erythema or excoriations, or both, seems to be associated with high percutaneous absorption. This association has been suggested previously (Robertson & Maibach, 1982). In the present patient series, the smallest rise in serum cortisol associated with abnormal adrenocortical function was 465 nmol/l. The clinical importance of absorption is seen in suppressed adrenal function in five of 13 children tested and in the development of Cushing's syndrome in one child.

To minimize the systemic side-effects of hydrocortisone in patients in whom high absorption has been found, it may be advisable to apply hydrocortisone cream in the morning in an attempt to mimic the physiological secretion of cortisol, as we did with Patient 4.

In patients investigated with a 24 h absorption test the absorption of hydrocortisone could be detected reliably by taking three serum samples before, and 2 and 4 h after, application respectively. The 4 h absorption test was developed on the basis of these findings, and can detect reliably the percutaneous absorption of hydrocortisone. This test, in addition to the monitoring of adrenocortical function and growth, is recommended for infants with chronic severe skin disorder requiring long-term treatment with topical glucocorticoids. High percutaneous absorption can be interpreted as a risk factor for systemic side-effects. On the other hand, the risk of systemic side-effects seems to be very small in older children and in infants in whom the involved skin areas are small or mildly affected.

## ACKNOWLEDGMENTS

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# Challenge reactions in atopic dermatitis after percutaneous entry of mite antigen

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## SUMMARY

We succeeded in reproducing an eczematous lesion on the apparently normal skin of a patient with atopic dermatitis by scratching and continuous application of an ointment containing ferritin-labelled mite antigen.

Percutaneous entry of mite antigen was demonstrated in skin biopsies by Fe-staining. Scratched skin first showed an urticarial reaction typical of type I allergy which later changed into an eczematous reaction typical of type IV allergy. This change was also shown histologically.

Atopic dermatitis (AD) is a disease associated with type I allergy (Yamura, 1980; Gondo, 1983), showing a positive reaction to multiple allergens in the immediate skin reaction test (Gondo, 1983; Rajká, 1961), particularly to mite antigen which was positive in about 90% of the AD patients tested.

Mite antigen is known to be a major antigen in respiratory atopic diseases such as bronchial asthma and allergic rhinitis; however, its antigenicity in AD remains questionable. The factors apparently against its antigenicity include the fact that the clinical form of AD is an eczematous reaction differing from the urticarial reaction of type I allergy, that a mite antigen is considered to be an inhalational antigen and the process whereby the antigen reaches the skin is still unclear in AD, and that the induction of AD with mite antigen is non-reproducible.

On the assumption that the *in vivo* absorption of mite antigen in AD is percutaneous, we investigated the role of this antigen in a patient with AD by applying an absorption ointment containing ferritin-labelled mite antigen to normal and scratched skin at sites with and without a previous history of a rash, and by studying the immunological, allergic and histological changes produced. On the basis of these results, a challenge test was attempted on patients with AD.

## METHODS

### *Subject*

A 25-year-old male patient with an 8-year history of severe AD and a family history of AD was tested. He had no history or evidence of other disease. The eruption was distributed mainly on

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TABLE 1. Results of immunological and allergy tests in our AD patient

| | | |
|---|---|---|
| Serum total IgE (RIST) (IU/ml) | | 560 |
| Antigen specific IgE (RAST) | | |
| House dust | | + |
| *D. farinae* | | + |
| *D. pteronyssinus* | | + |
| Serum histamine (ng/ml) | | 0·6 |
| Blood esosinophils (%) | | 12 |
| Blood basophils (%) | | 1 |
| Blood lymphocytes (%) | | 20 |
| T-cell subsets | OKT3 (%) | 84·4 |
| | OKT4 (%) | 53·9 |
| | OKT8 (%) | 31·7 |
| | OKT4 (ratio) | 1·7 |
| Immediate skin test (standard) | Mite | + |
| | House dust | + |
| | Household insect mix | + |
| | All other standards | − |

the extensor surfaces of the limbs and the dorsal side of the body, also involving the face and the head, with the total affected area exceeding 80%.

Results of the main immunological and allergy tests are shown in Table 1, while the types and concentrations of antigens used as standards are summarized in Table 2.

From these test results, and the fact that the patient complained of intense itching when in a dusty place, involvement of type I allergy due to mite antigen was deduced. High IgE RAST scores were obtained with *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*.

TABLE 2. Types and concentrations of antigens used

| Allergens | Protein concentration (PNU/ml) | Dilution | Injected volume (ml) |
|---|---|---|---|
| House dust | 20 000 | 1 : 2500 | 0·02 |
| Mite | 5000 | 1 : 2500 | 0·02 |
| Household insect mix | — | 1 : 1250 | 0·02 |
| Epidermal mix | 9000 | 1 : 2500 | 0·02 |
| Mold mix | 20 000 | 1 : 2500 | 0·02 |
| Japanese cedar | 1000 | 1 : 2500 | 0·02 |
| Ragweed mix | 40 000 | 1 : 2500 | 0·02 |
| Orchard grass | 40 000 | 1 : 2500 | 0·02 |
| Sagebrush mix | 40 000 | 1 : 2500 | 0·02 |
| Cow's milk, whole | 20 000 | 1 : 500 | 0·05 |
| Wheat, whole | 20 000 | 1 : 500 | 0·05 |
| Soybean, whole | 20 000 | 1 : 500 | 0·05 |
| Rice, whole | 8000 | 1 : 500 | 0·05 |
| Corn, whole | 20 000 | 1 : 2500 | 0·02 |
| Buckwheat | 20 000 | 1 : 2500 | 0·02 |

PNU: Protein nitrogen unit.

### *Ferritin-mite conjugate*

Ferritin-mite conjugate was prepared according to the toluene-2-4-diiscoyanate (TC) method. Briefly, lyophilized powdered *D. farinae* (6·2 mg) and ferritin type I from horse spleen (Sigma Co. Ltd, St. Louis, U.S.A.) were mixed, purified once with cadmium sulphate (final concentration 5%) and dialysed against 0·05M phosphate buffer (pH 7·5) for 24 h at 5°C. The binding capacity of the ferritin-mite conjugate was confirmed by electron microscopy, and the concentration of *D. farinae* in the solution was increased to 1 : 10 with minicon (Amicon Co. Ltd, Danver, U.S.A.). The ferritin-mite conjugate was then mixed with an absorption ointment consisting of 40% white petrolatum, 36·3% distilled water; 18% cetanol, 5% sorbitansesquiorate, 0·5% polyoxyethylenelauryl alcohol ether, 0·1% methyl *p*-hydroxybenzoate, and 0·1% propyl *p*-hydroxybenzoate.

### *Skin test*

One hundred percent *D. farinae* dry powder, 10% *D. farinae* absorption ointment, 2% *D. farinae* absorption ointment and 1% ferritin-*D. farinae* conjugate were applied separately to normal and scratched skin on the extensor side of the forearm. Scratching was done in a criss-cross pattern, using a two-needle steel prong, to an extent which did not cause bleeding. Immediate skin reactions were recorded after 15 min, and subsequent readings made after 7, 24 and 48 h using a closed patch test. The assessment of an immediate reaction based on the presence of a weal and its diameter, and a delayed type reaction was assessed according to the standard criteria for a patch test. This skin test was also performed in five other patients fulfilling the diagnositc criteria for AD.

### *Challenge test*

Two percent *D. farinae* absorption ointment, which had produced a positive skin reaction, was applied twice a day, (morning and evening) to the extensor surface of the forearm where there was persistent dermatitis, and to the flexor surface where there was no history of dermatitis. On the first two occasions, the whole site to be tested was scratched, while in subsequent tests the upper half was scratched and the lower half was left intact. In addition, the test site was covered with cotton gauze to which the patient had previously shown no irritant or allergic reactions.

### *Immunological tests*

After allergen challenge, blood eosinophil and basophil counts and serum complement titre were determined. Serum histamine, measured by fluorometry using the method of Shore, Burkhalter and Cohn (1959), total serum IgE measured by RIST (Miyamoto, Ogawara & Nishimura, 1972), and mite antigen specific IgE measured by RAST (Okuda, Usami & Unno, 1975) were also determined.

### *Histology*

Biopsies were taken from the challenge test areas 15 min, and 7 and 48 h after allergen challenge. Tissue samples were stained with H & E or Toluidine blue for observation of allergic responses, and with Fe to detect percutaneous penetration of mite antigen.

## RESULTS

### *Skin test reactions*

Results of the skin test are given in Table 3. After 15 min, an urticarial reaction was noted at the scratched sites treated with 10%, 2% and 1% *D. farinae* ointments. The site treated with 100% *D. farinae* dry powder was negative. Results from the non-scratched sites were all negative. The positive reactions to the skin tests were accompanied by generalized, intense itching, which disappeared after about 30 min, as the skin reaction faded.

After 7 h, the scratched site treated with 100% *D. farinae* dry powder developed intense itching and oedematous erythema indicative of a delayed reaction. A similar reaction was also observed at the sites treated with 10%, 2% and 1% ointments. The non-scratched sites remained negative.

TABLE 3. The results of skin test with mite antigen in the patient with AD

| | Scratched | | | Non-scratched | | |
|---|---|---|---|---|---|---|
| | *D. farinae* | | | *D. farinae* | | |
| Time | 100% | 10% | 2% | 100% | 10% | 2% |
| 15 min | — | EW | EW | — | — | — |
| 7 h | E | E | E | — | — | — |
| 24 h | E | E | E | — | — | — |
| 48 h | EP | EP | EP | — | — | — |

E, erythema; W, weal; P, papules; —, no reaction.

After 24 h, the positive reactions on all the scratched sites had faded, leaving slight erythema and persistent itching. The non-scratched sites remained negative. Forty-eight hours after challenge the non-scratched sites were still negative. Itching and erythema persisted on the scratched sites and there was an eczematous reaction at the sites treated with the 100% and 10% *D. farinae* (Fig. 1). Results from five other AD patients are summarized in Table 4.

Patch tests and scratch patch tests using the absorption ointment were performed on six AD patients and 10 non-atopic healthy controls. All the results were negative.

### *Challenge reactions*

The changes in challenge test reactions with time are shown in Table 5. An urticarial reaction was observed on both extensor and flexor surfaces 15 min after challenge, similar to the reaction in the skin test. The urticarial reaction became weaker after the fourth application, and was almost negative after the fifth, itching only. This change was more pronounced on the extensor surface.

Reactions similar to those noted in the skin test were also observed 7 h after challenge (before the second application) on both extensor and flexor surfaces. This apparent delayed reaction became milder after the second application and disappeared completely by the third.

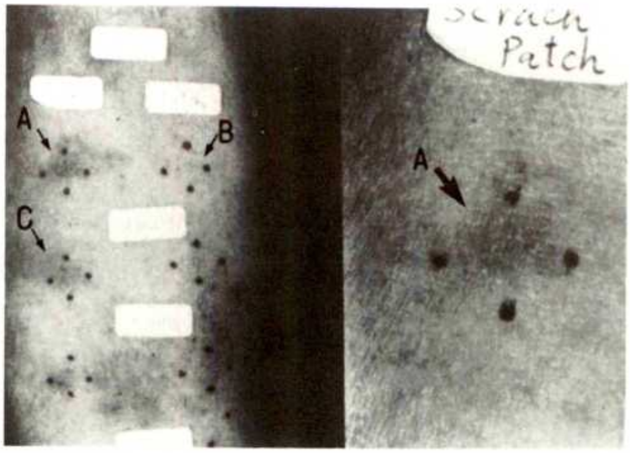

FIGURE 1. Skin reaction after 48 h with *D. farinae* antigen. An eczematous reaction with papules was noted on scratched skin treated with 100% *D. farinae* (A) and 10% *D. farinae* (C). All treated sites on non-scratched skin were negative (B).

TABLE 4. The results of skin test with 2% *D. farinae* antigen in five AD patients

| Case no. | Scratched | | | | Non-scratched | | | |
|---|---|---|---|---|---|---|---|---|
| | 15 min | 7 h | 24 h | 48 h | 15 min | 7 h | 24 h | 48 h |
| | EW | E | E | EP | | | | |
| 1 | + + | + | + | + + | — | — | — | — |
| 2 | + + | + | + | + + | — | — | — | — |
| 3 | + + | + | + | + + | — | — | — | — |
| 4 | + + | + | + | + − | — | — | — | — |
| 5 | + + | — | — | — | — | — | — | — |

E, erythema; W, weal; P, papules; —, no reaction.

Thereafter, both surfaces remained negative. However, an eczematous reaction (oedematous erythema with red papules) appeared on the scratched extensor surface before the ninth application (on the 5th day). No such reaction could be detected on the non-scratched area, or on the scratched flexor surface. When the bandage was removed to see if eczema could be reproduced in the natural state, scratching occurred in response to the persistant itch, resulting in an appearance resembling established AD on the scratched extensor surface, but not on the other sites. An urticarial reaction no longer appeared after the fifth application of 10% *D. farinae* absorption, but reappeared when 50% *D. farinae* absorption ointment was used.

TABLE 5. Response to the challenge test with mite antigen

| Challenge no. | Time after challenge | Extensor surface | | Flexor surface | |
|---|---|---|---|---|---|
| | | Scratched | Non-scratched | Scratched | Non-scratched |
| 1 | 15 min | Urticarial reaction | ND | Urticarial reaction | ND |
| | 7 h | Erythema only | ND | Erythema only | ND |
| | 24 h* | Erythema only | ND | Erythema only | ND |
| 2 | 15 min | Urtical reaction | ND | Urticarial reaction | ND |
| | 24 h* | Erythema only | ND | Erythema only | ND |
| 3 | 15 min | Urticarial reaction | — | Urticarial reaction | — |
| | 24 h* | Erythema only (±) | — | Erythema only | — |
| 4 | 15 min | Urticarial reaction (±) | — | Urticarial reaction | — |
| | 24 h* | Erythema only (±) | — | Erythema only (±) | — |
| 5 | 15 min | No urticarial reaction | — | Urticarial reaction (±) | — |
| | 24 h* | Erythema only (±) | — | — | — |
| 6 | 15 min | Erythema only (±) | — | No urticarial reaction | — |
| | 24 h* | Erythema only (±) | — | — | — |
| 7 | 15 min | Erythema only (±) | — | — | — |
| | 24 h* | Erythema only (±) | — | — | — |
| 8 | 15 min | Erythema only (±) | — | — | — |
| | 24 h* | Eczematous reaction | — | — | — |
| 9 (open) | 15 min | Eczematous reaction | — | — | — |
| 12 (open) | 24 h* | Eczematous reaction (+ +) | — | — | — |
| 13 (open) | 15 min | Eczematous reaction (+ + +) | — | — | — |

* Before next challenge; —, negative reaction; ND, not done (see Methods); (±), weak reaction; + +, moderate reaction; + + +, intense reaction.

*Immunological tests*

Results of the immunological tests before and after the allergen challenge test are shown in Table 6. The blood eosinophil count decreased immediately after challenge, the serum histamine level began to decrease reaching a trough after 48 h and then increase to a value similar

TABLE 6. The results of immunological tests before and after challenge

| Time after challenge | Eosinophils % | Basophils % | Serum histamine ng/ml | Serum total IgE U/ml | Mite antigen specific IgE U/ml | CH50 IU/ml |
|---|---|---|---|---|---|---|
| Pre-challenge | 9 | 1 | 0·64 | 180 | 17·5 | 31·6 |
| 15 min | 3 | 1 | 0·79 | 130 | 17·5 | 31·1 |
| 7 h | 4 | 0 | 0·56 | 160 | 17·5 | 30·8 |
| 24 h | 1 | 1 | 0·47 | 120 | 17·5 | 33·2 |
| 48 h | 4 | 0 | 0·26 | 140 | 16·0 | 24·6 |
| 2 weeks | 10 | 2 | 0·60 | 360 | 17·5 | 27·3 |

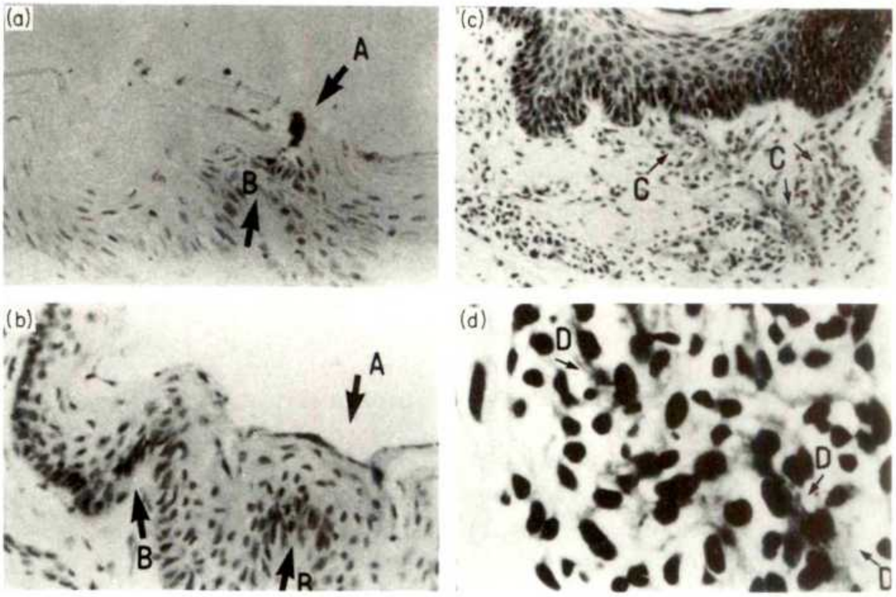

FIGURE 2. Histological features of challenge reaction with ferritin-2% *D. farinae* conjugate. (a) and (b) Fe-staining 15 min after challenge. Fe-positive material appeared mainly in the skin creases (A) extending from the stratum corneum to the epidermis (B). (c) and (d) Toluidine blue-Fe staining. Abundant Fe-positive material is present in the epidermis (C) and many mast cells with degranulation (D) coincide with this.

to the pre-challenge level within a few days of the completion of the challenge test. Total serum IgE after the challenge test was increased to about double the pre-challenge level. Mite antigen specific IgE showed a decrease 48 h after challenge. Serum complement (CH50) reached a peak after 48 h, followed by a decrease with substantially lowered values persisting for several days.

### *Histology*

*H & E staining*. There was evidence of an urticarial reaction 15 min after testing with oedema of the upper dermis and cellular infiltration. After 7 h, the oedema of the upper dermis was reduced and there was increased cellular infiltration by eosinophils. Changes including acanthosis and liquefaction occurred in the epidermis after 48 h, with further reduction of oedema and dense cellular infiltration by eosinophils, indicating an eczematous rather than an urticarial reaction.

*Toluidine blue and Fe staining*. After 15 min, Fe-positive material was present from the stratum corneum through the epidermis, mainly involving skin creases. Staining was faint in the dermis but toluidine blue-positive mast cells and their degranulation products were observed (Fig. 2). After 48 h, abundant Fe-positive material was observed in the dermis, together with many degranulating mast cells. Similar features were observed in part of a follicle. These findings indicate that percutaneous entry of mite antigen caused urticarial reactions of type I allergy initially, which gradually changed to eczematous reactions later.

## DISCUSSION

The rash appearing in AD is an eczematous reaction, which is different from the urticarial reaction of type I allergy. The initial symptoms of AD are said to be itching and an urticarial reaction, (Yamura, 1980) but the rash usually observed in practice is an eczema. However, the eczema, rather than being the primary eruption, is likely to be the result of various repeated stimuli, combining reactions including type I and type IV allergy with a primary irritant response to a combination of physical, chemical and mechanical factors, including scratching due to persistent itching. Our experimental results support this proposal. Following the percutaneous challenge with mite antigen, a type I reaction occurred in our patient. An eczematous type IV reaction occurred on repeated challenge. We suggest that this change occurred either through the action of a blocking antibody specific for mite antigen, (probably caused by hyposensitization and with the suppressive antibody acting against type I allergy or type IV allergy), or by late reactions acting as part of a feedback system.

The route of penetration of mite antigen is still unclear in AD. In the present study we have demonstrated percutaneous entry to be one possible route. In the skin biopsies performed after the application of ferritin-mite conjugate absorption ointment, the pattern of iron staining moved from the stratum corneum to the epidermis and then to the dermis. Toluidine blue and Fe staining revealed numerous mast cells and their degranulation products at the Fe-positive sites. Kanda *et al.* (1984) found the localization of mite antigen in the skin by means of a fluorescent antibody method, and observed the intra-epidermal aggregation of OKT6 positive cells and infiltration into the dermis in this region. These findings all demonstrate that mite antigen can invade percutaneously. From the Fe staining results, this invasion is considered to be through the skin creases or follicles, and toluidine blue staining confirmed allergic reactions in this region.

The skin in AD is rough, dry and excoriated. We prepared scratched skin sites in areas of previously normal skin in AD patients, and mite antigen was applied continuously to these scratched sites, ultimately resulting in the successful reproduction of eczema lesions. This response only appeared on the extensor surface and no lesions appeared on scratched skin on the flexor surface. The results suggest that the reproducton of AD requires various conditions, namely, the site of the normal distribution of the lesions which varies in different cases (the extensor rather than the flexor surface in our patient), suitable conditions for allergen penetration (scratched or dry skin and/or site of inflammation), and a sufficient amount of antigen and its continued administration.

From the above evidence, the antigenicity of mite antigen in AD was confirmed, which seems to give an important clue for elucidating the pathogenesis of AD.

The facts that high levels of serum IgE immune complexes are encountered frequently in AD (Ota, Gondo & Tokuda, 1983; Gondo *et al.*, 1985), that mite antigen specific IgE in these complexes correlates with mite antigen specific IgE in serum (Gondo *et al.*, 1985), that a delayed reaction is observed frequently following a skin test with mite antigen in addition to the immediate reaction, and that CH50 decreases during challenge tests with mite antigen are difficult to explain in terms of type I allergy. In AD, an allergy not only of type I but of, for example, type III may also be involved to some extent. Increased circulating OKT4: OKT8 ratio, decreased OKT8-positive lymphocytes (Gondo, 1983) and increased tissue OKT8-positive lymphocytes (Kanda *et al.*, 1984) have been reported, while decreased reactivity against DNCB, PHA, SK/SD and *Candida* (Koitabashi *et al.*, 1983) and susceptibility to infection with viruses (Fritz *et al.*, 1980) and bacteria (Walsh *et al.*, 1981) have also been observed, suggesting abnormal cellular immunity (Grove, Reid & Forbes, 1975). Further studies on the mechanism of these changes are needed for a better understanding of AD.

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***Case Reports***

# Secondary syphilis mimicking Sweet's syndrome

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## SUMMARY

We describe a patient with secondary syphilis and facial skin lesions which resembled Sweet's syndrome clinically and histologically. We suggest serological tests for syphilis in patients with Sweet's syndrome.

Syphilis, and especially secondary syphilis, may mimic many dermatological conditions (Rhodes & Luger, 1979). This mimicry is not only confined to the clinical appearance of lesions, but can extend to the histopathological picture (Abell, Marks & Wilson-Jones, 1975).

We report a patient who had secondary syphilis and facial skin lesions, which resembled lesions of Sweet's syndrome on clinical and histopathological grounds. To our knowledge this phenomenon has not been documented previously.

## CASE REPORT

A 25-year-old female presented with facial lesions which had been present for 2 weeks.

On examination, there were three annular lesions. The periphery consisted of confluent erythematous papules and the central part showed oedema and brownish hyperpigmentation. Small ulcers were noted on the tongue and labia majora.

Biopsies from a peripheral papule and the central area were processed in routine fashion for light microscopy and stained with haematoxylin and eosin.

The epidermis of the papuar lesion was acanthotic and infiltrated by neutrophils. A diffuse cellular infiltration with a nodular tendency occupied the upper two thirds of the dermis but was perivascular in the lower third. Nodules consisted of centrally located neutrophils, showing leukocytoclasia, and peripheral lymphocytes and histiocytes. These collections of neutrophils appeared abcess-like. Neutrophils were virtually absent in the lower dermis, and only lymphocytes and histiocytes were noted. Extravasated red cells and melanophages occurred in the upper dermis. Plasma cells were absent. Endothelial proliferation was noted but fibrinoid necrosis of vessels and microthrombi were not seen.

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Biopsy from the centre of the lesions showed a superficial and deep lympho-histiocytic infiltrate. Neutrophils were entirely absent. No plasma cells were seen. Many melanophages occupied the upper dermis.

The white cell count was $15 \times 10^9$/l with 71% neutrophils. The VDRL test was positive at a titre of 1:256. The FTA-ABS (fluorescent treponemal antibody-absorption) test was positive. A diagnosis of secondary syphilis was made and the patient treated with a weekly injection of 2·4 million units benzathine penicillin over a period of 4 weeks.

Follow-up at 4 weeks showed healing of skin and mucosal lesions without scarring. The VDRL titre was 1:16.

## COMMENT

This patient clearly had secondary syphilis. The eruption on the face resembled the lesions of Sweet's syndrome on clinical and histopathological grounds.

This situation raises two possibilities. Firstly, that secondary syphilis is a cause of Sweet's syndrome. Clinically and histologically the lesions resembled those of Sweet's syndrome. A neutrophilia occurs in Sweet's syndrome, but also in secondary syphilis. A great variety of conditions may 'cause' Sweet's syndrome (Storer *et al.*, 1983), and syphilis, or more specifically, *Treponema pallidum* infection, could be one of them. Secondly, it is possible that secondary syphilis is an imitator of Sweet's syndrome. The simultaneous appearance of the skin and mucosal lesions and the rapid response to penicillin treatment militates in favour of this. It is well recognized that syphilis is a great imitator, not only clinically, but also histologically.

Until further evidence is available, we suggest that serological tests for syphilis should be done in patients with Sweet's syndrome.

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# Giant orf in a normal individual

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## SUMMARY

A case of giant orf granuloma is described in a normal 12-year-old child.

## CASE REPORT

A 12-year-old boy, who lived on a farm, suffered a 'rope burn' on the left side of his cheek and neck. Three weeks later an inflamed spot developed in a scab on the cheek, which rapidly developed into a pedunculated mass (Fig. 1). The mass was thought originally to be a pyogenic granuloma, but closer questioning revealed that the boy had a pet sheep with which he had had close facial contact. The sheep was probably suffering from orf at the time. It was, therefore, presumed that orf virus had been acquired from the sheep and had been inoculated into the residual scabs of the 'rope burn'.

The tumour was firm, not painful and bled easily. It measured 7·5 cm by 5 cm and was attached to the skin by a 2 cm round base. There was some thickening of the sub-epidermal layer of the cheek immediately below the tumour, but there was no evidence of involvement of the inside of the cheek.

Superficial scrapings failed to reveal orf virus on electron microscopy, but orf was still considered to be the most likely diagnosis. Spontaneous regression could have been expected, but the tumour wobbled and the child was unable to go to school as he was being mocked. At surgery the tumour bled somewhat, but was easily excised by transection at skin level; the base was cauterized and a non-adherent dressing applied. The skin healed well without scarring and 2 months later the only residual sign was a slight pink blemish. Two years later the boy is well and the skin looks normal.

Histological examination showed a polypoid mass of inflamed granulation tissue with areas of marked oedema and some fibroblastic activity. The overlying epidermis was mildly papillomatous and focally ulcerated, and displayed thickening, spongiosis and a neutrophil polymorph infiltrate.

The lesion thus bore a superficial similarity to an inflamed pyogenic granuloma, but lacked its flattened epidermis, prominent vascularity and so-called epidermal collarette. Intracellular

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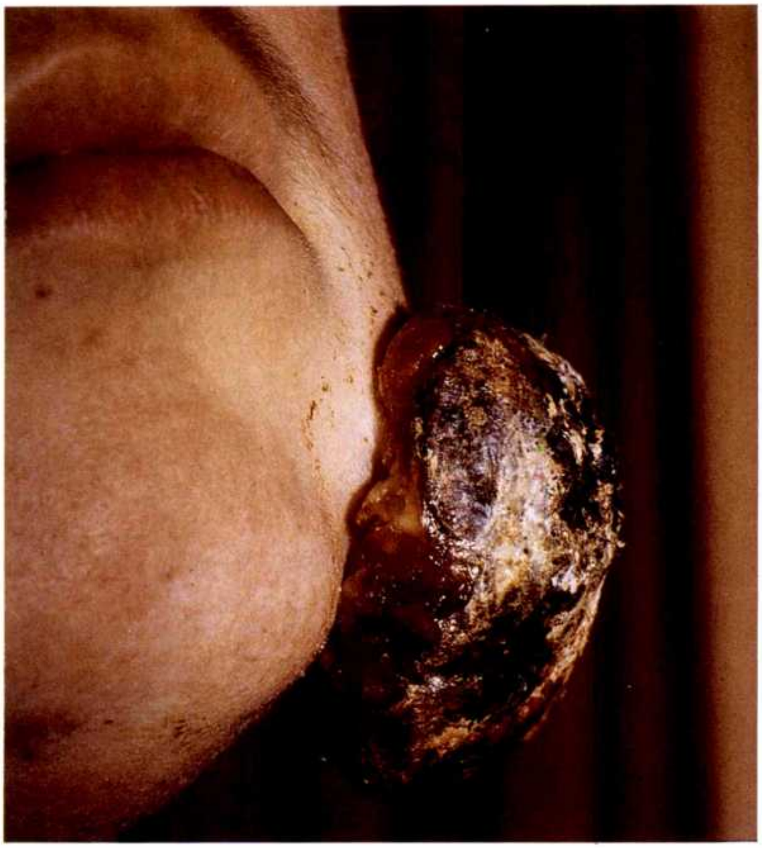

FIGURE 1. Clinical appearance of giant orf lesion.

inclusion bodies were sought but not identified. They are usually seen in histological sections only in the early stages of orf skin infections.

Material from a cut surface examined with an electron microscope at the Bristol Public Health Laboratory revealed numerous particles typical of the orf virus (Nagington, Newton & Horne, 1964), thus confirming our suspicion that the tumour was a granulomatous response to the orf virus.

## DISCUSSION

Granulomatous lesions, similar to the one in our patient, have been described in a patient with lymphoma (Savage & Black, 1972), associated with atopic dermatitis (Dupre *et al.*, 1981), and protruding from the ear of a normal person (Nagington, 1983, personal communication). Persistent infection in a ram has been described with numerous papillomatous growths (Greig, Linklater & Clark, 1984).

The boy had no generalized rash, erythema multiforme nor other unusual manifestations of infection by the orf virus (Wilkinson, 1977). There was no clinical evidence of general ill-health nor any reason to suppose that the child was immunologically compromised. The reason for the curious granulomatous overgrowth is thus obscure, except that the site of infection was a healing laceration.

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# Blisters over burn scars in a child

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## SUMMARY

We present the case of an 11-month-old baby with a burn that healed normally over a month, but subsequently developed successive crops of blisters over the scar. There were no changes in his general condition. Clinical, immunological, histological and ultrastructural studies provided a diagnosis of mechanical dermatitis produced by microtrauma. Electron microscopy studies revealed the sub-epidermal nature of the blisters and the presence of underlying fibrin deposits.

It is usual to observe blisters over early burns or burn scars, either recent or already atrophied. Such lesions are usually attributed to unavoidable minor trauma such as rubbing or friction. Ackerman (1978) considers that this phenomenon is due to a false adherence between epidermis and dermis, and Wong and MacDonald (1982) believe that the deposition of fibrin, as occurs in frictional lesions, can also be a contributing factor to blister formation.

We have studied a boy who suffered burns produced by boiling milk. When the burned areas were epithelialized completely, bullous lesions, which could be attributed to friction caused by the dressings, appeared over the scar.

## CASE REPORT

The patient was an 11 month-old boy with no previous personal or familial history of relevant illnesses. His general condition and psychomotor development were good and he had been vaccinated correctly. He was admitted to our burn unit 1 h after being burned with boiling milk. The burn encompassed 30% of his body surface reaching second degree at many points, and with a splash distribution over the head, neck, chest and arms. Sphacellous skin rests were removed and 1% silver sulphadiazine was applied for the first 15 days. Subsequently, he was treated with 5-nitro-2-furaldehyde semicarbazone up to complete healing. The dressings were

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This case was reported at the 1st Irish-Catalan Symposium, Belfast, Ireland in October 1985.

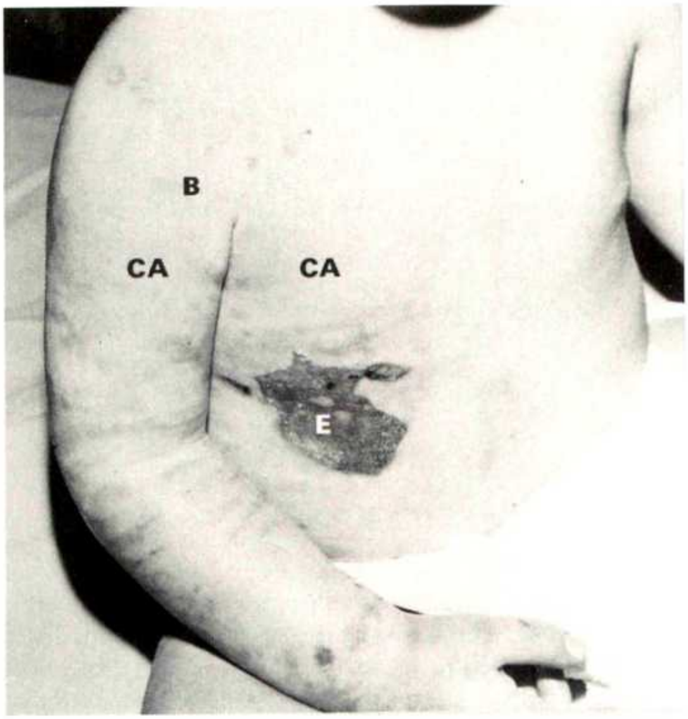

FIGURE 1. Blisters over burn scars 43 days after the burn. CA, cicatricial area; E, erosion; B, bulla.

always occlusive, and were changed once a day. During the first month he was treated with fluids, electrolytes, plasma and analgesics.

Twenty-eight days after admission, the burns were fully epithelialized, but seven round erosions measuring 0·5–2 cm in diameter appeared on the scar areas. These lesions were scattered over the forehead and the arms. In succeeding days, crops of lesions with similar features appeared regularly. One week after the observation of the first erosions, we observed the presence of small bullous elements (Fig. 1) with no surrounding inflammatory base, a flaccid aspect and serous content. These bullous lesions rapidly underwent erosion and were usually located in zones of friction. The Nikolsky's sign was positive on the cicatricial area but negative on healthy skin. One month after the first observation of the erosive lesions, the number of bullous lesions diminished rapidly, and this coincided with the cessation of the dressings. This allowed the patient to be discharged from hospital.

Three months later, mild erosions were still present in points of maximal friction including the waist and axillae. The lesions had disappeared completely 6 months after onset.

### *Biochemistry, histology and electron microscopy*

A full blood count and measurements of ESR, glucose, urea, electrolytes, creatinine, lipids, blood proteins, transaminases, and alkaline phosphatase, and clotting tests were all normal. Tests for anti-epithelial, anti-dermo-epidermal junction and pemphigus-like antibodies were negative. Cultures of the serous content of the blisters and of the erosive lesions were also negative.

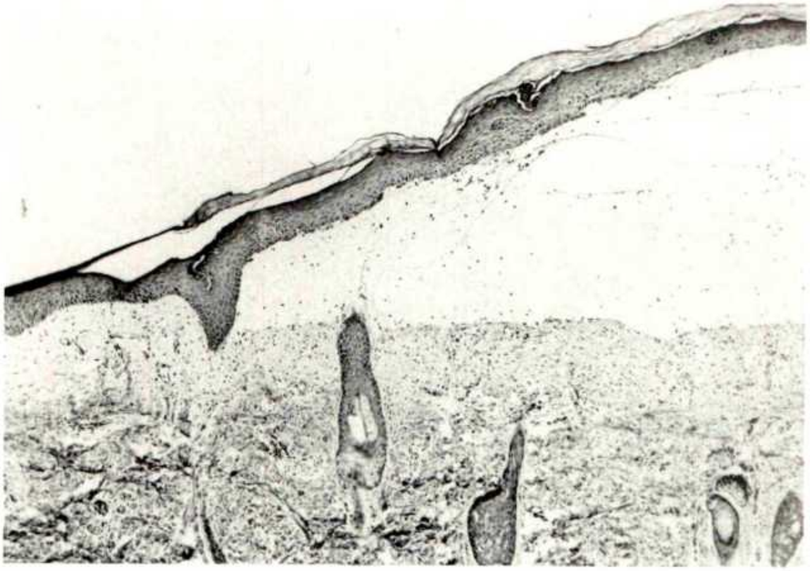

FIGURE 2. Subepidermal bulla (original × 40).

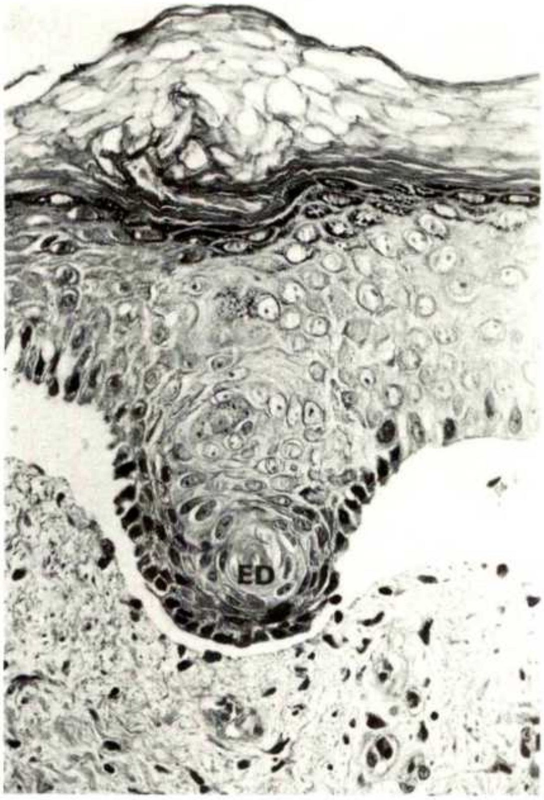

FIGURE 3. Eccrine duct (ED) showing 'dissected' appearance (original × 100).

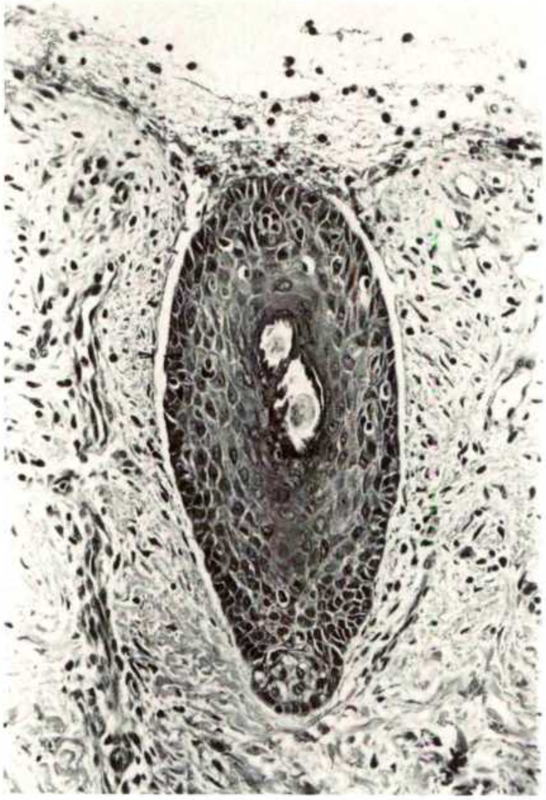

FIGURE 4. Cleft between dermis and hair follicle (original × 100).

Histologically, sub-epidermal blisters were observed at low magnification (Fig. 2). The dermo-epidermal split extended only along the cicatricial segment and reached the accessory anexae (eccrine ducts and pillous follicles) which were dissected clearly (Figs. 3 and 4). Electron microscopy showed that the roof of the blisters comprised the epidermis with the basal membrane and part of the underlying dermal collagen, between fibrin deposits.

## DISCUSSION

The appearance of blisters on a new scar area cannot be attributed to the topical treatment with semicarbazone and silver sulphadiazine since these drugs are usually applied in our burn unit, and no similar cases have been observed among approximately 2000 newly burned patients admitted to the unit over 10 years.

Initially, the presence of the Nikolsky's sign on the scar suggested the possibility of a pemphigus provoked by the burn (Chorzelski *et al.*, 1971). However, the fact that the bullous lesions did not extend beyond the burned area at any point, and the presentation after a recent but nevertheless complete cicatrization, militate against pemphigus. Also, pemphigus in a child is very uncommon (Saban *et al.*, 1985). Later, this possibility was excluded definitively by the histological findings and the negative direct immunofluorescence. Anti-pemphigoid and anti-pemphigus antibodies were not detected, although they are occasionally found at low titres in burned patients up to 2 weeks after the burn (Thivolet & Beyvin, 1968; Ablin *et al.*, 1969; Trifthauser, Beutner & Wypych, 1970).

The lack of evidence for a larvate form of genetic-dermatosis from a careful family history, the absence of evidence of an autoimmune or infectious disease, and the patient's age and his generally good health, as well as the limitation of the lesions to the burned areas, restricted the diagnostic possibilities to those bullous processes with histological evidence of sub-epidermal blisters without a significant underlying inflammatory infiltrate. Electron microscopy established that the blisters arose within the papillary dermis. The roof of the blisters consisted of the whole dermis and all the elements of the dermo-epidermal junction.

An identical histological picture has been observed in blisters in skin grafts, in the areas where the skin is obtained as well as in those where it is implanted (Baran, Juhlin & Brun, 1984). In the three patients reported by these authors, however, there was no evidence of friction being involved. An identical histological picture has been observed in the dermal forms of dystrophic bullous epidermolysis (DFDBE) (Pearson, 1962) which, in addition, are accompanied by a reduction in the number of anchoring fibrils (Briggaman & Wheeler, 1975; Hashimoto *et al.*, 1975; Hashimoto *et al.*, 1976 a,b). Friction is a well known experimental inducer of blister formation, as are suction or local application of heat. (Kero, 1984; Kiistala, 1968; Tidman & Eady, 1983). We believe that in the present case the dressing acting as a source of friction, and the presence of fibrin, provoked production of bullae. The fractures of newly formed dermal collagen fibres were probably a secondary phenomenon. The possibility of a larvate form of DFDBE can be excluded on grounds of the spontaneous healing when the recently epithelialized area was left uncovered, and the absence of relapse after one year of follow-up.

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## *Obituary*

# Hermann Pinkus (1905–1985)

Hermann Pinkus followed his celebrated father, Felix Pinkus of Berlin, anatomist and clinician, in a dermatological career, but in the United States of America, becoming celebrated himself, and accumulating many honours in his long and active life. To the casual observer, Hermann might give little hint of great erudition or distinction, for he had a quiet, friendly manner, and lacked the varnish of self-advertisement. But when you got to know him you realized that he was unusually knowledgeable, and that it was his habitual modesty that concealed his exceptional qualities from casual inspection. It was this modesty that enabled him to treat anyone, however junior, as his intellectual equal, provided only that he or she was actuated by a desire to learn and to understand. Modest he may have been, but there was nothing flabby about him. He was compact, both physically and mentally, and had a core of steel, though this was formed like a spring, to give strength and resilience, not to be used as a rapier.

A rugged constitution and a determination to succeed brought him through some turbulent years in the history of the world, and his eventual success owed much to his persistence, energy and hard work. Having qualified M.D. in Berlin in 1930, with a thesis based on tissue culture, and then trained in dermatology in Professor Jadassohn's Department at Breslau, he decided he must leave Germany, because of the political climate, and he arrived in the United States in 1934, a refugee without money, work or prospects. Through the help of Marion Sulzberger he found a job in tissue culture, as Research Fellow in the University of Michigan, at Ann Arbor, and from this beginning he progressed, via a Fellowship in Dermatopathology at Wayne County General Hospital under Professor Franz Blumenthal, private practice, and a part-time teaching post at the Department of Dermatology at Wayne State University, Detroit, to become, in 1960, a full-time Professor and Chairman of that Department.

In the early years of his career in the United States he was supported and encouraged by Hilda Hensel, herself a doctor, who, in 1935, came from Germany to marry him. They settled at Monroe, Michigan, 40 miles south of Detroit. Early in the morning Hermann would set out to drive to hospital in Detroit, and in the evening he would drive home to his private practice, reporting on the slides that had been processed during the day by his housekeeper, from the biopsy specimens that had arrived by post. Such a gruelling routine can have given little time for any sort of private life.

Although Hermann Pinkus considered himself a self-taught dermatopathologist, his father's expertise must have given him a head start, and histopathology was a routine method of investigation at Breslau, where he was taught by Walter Freudenthal, in addition to the Fellowship in Dermatopathology with Franz Blumenthal. He was a minute observer, confident in his own powers, and able to draw just conclusions from his wide experience. His publications, and especially the three editions of the book on Dermatohistopathology, written with A.H. Mehregan, revealed an intimate personal knowledge of the subject. Working with him, it was clear that the reference he consulted most often in tackling a problem was the one kept inside his head, composed of his own observations.

The love of anatomy that Hermann inherited from his father was the mainspring of all his work; his mother's gift was a delight in the natural world, the joy of watching things grow, and develop. This sense of change wrought by time converted his father's three-dimensional view of the skin into Hermann's four-dimensional one, a vantage point that made him such an outstanding dermatopathologist. Hermann's dynamic view of biology had begun as a student in Berlin, as a result of tissue culture work under Rhoda Erdmann, while Hans Spemann's ideas on cell and tissue interaction worked in Hermann's mind throughout his life.

If the skin was the limited arena in which Hermann was seen to exercise his talents, his ability to do so depended on a wide and constantly developing general knowledge of biology, served by a tenacious, but accessible, memory, and guided by an exceptionally well developed sense of propriety. When the 'orthodox' picture of actuality existing in his own mind confronted the 'unreasonable' idea of others, he was stimulated to try and disprove them experimentally. Thus, the study of stripping the skin with cellophane tape originated from his disbelief that lymphocytes migrated into the epidermis and became keratinocytes, an idea that had been proposed to explain the apparent dearth of mitoses in the basal cells, believed to be insufficient to make good normal wear and tear. But the frantic mitotic activity induced by stripping left no doubt that the epidermis was capable of replacing itself, while a scale drawing of the epidermis showed Hermann that the observed rate of mitosis under resting conditions was perfectly adequate, because 25 basal cells underlay one horny cell.

Many of Hermann's discoveries were based on anatomy. One that gave him much pleasure was the trichodiscoma, a benign tumour of the *Haarscheibe*, or hair disc, a structure that had been described by his father. Hermann's attention had been engaged early on by Freudenthal's description of senile or solar keratosis. What, Hermann wondered, were the orthokeratotic columns of cells in the corneum doing? They seemed to be derived from the so far unaffected sweat ducts and hair follicles. Pursuing and developing this idea over many years led Hermann to recognize the acrosyringium and the acrotrichium as distinct anatomical entities, to discover the eccrine poroma as a tumour of the acrosyringium, to describe trichilemmal keratinization in the isthmus of the anagen hair follicles, and to show that wens were not sebaceous, but trichilemmal cysts.

His experience with tissue culture led him to view the epidermis as 'an intricate combination of biologically independent and interacting populations of cells' rather than a uniform tissue. It also led to the idea of symbiosis between these cell populations, while his concept of their potential for growth underlay his theory of the development of the adnexal tumours of the skin.

When he encountered a co-operative gentleman bearing a variety of tumours on his back, Hermann's anatomical self was led to describe the pre-malignant fibro-epithelioma, from which basal cell epithelioma developed; but the spirit of Hans Spemann went on to consider the influence of the stroma on the tumour, and to evolve the idea that basal cell epitheliomas are dependent upon the stroma for their survival. Latterly, Hermann showed that basal cell epithelioma implanted into rat uterus reverted to stratified squamous epithelium.

The description of alopecia mucinosa depended on Hermann's retentive memory, for it comprised observation of seven cases over seven years. The well prepared mind recognizes new things clearly, and Hermann's mind was exceptionally well prepared, by his heredity, his upbringing, the use that he made of his own talents, and, above all, by his relentless rumination over what had gone before, turning his ideas over perpetually in his mind, comparing them with the ideas of others, testing, modifying, being led on to new ideas of his own, constantly developing his view of the world of the skin. He was a living example of his father's dictum, that a man serves God best by using his talents constructively.

Hermann Pinkus was the Macfarlane Professor of Experimental Medicine at the Glasgow Royal Infirmary in 1973/74, during which visit he began to work on implanting basal cell epitheliomas into rat uterus, and delivered seminars that delighted pathologists, dermatologists, gynaecologists and plastic surgeons alike. The year that Hermann and Hilda spent in Glasgow will never be forgotten by those who experienced it, and for me it was a magic time; and when it as over, as my wife and I watched their plane disappearing high over Arran, the world temporarily came to an end for me.

Two years later Hermann Pinkus received an Honorary Doctorate of Law from Glasgow University. I watched him, standing in the Bute Hall, in his white tie, with his short, grey hair and neat moustache and spectacles, his face turned, like some inquisitive bid, towards Robert Goudie, Professor of Pathology, who was presenting him; while Robert, in the jocular fashion adopted on such occasions, said: 'Apart from being one of the few academics who is literally wrapped up in his subject, perhaps the most outstanding talent of Hermann Pinkus is in hiding his light under a bushel.' And that sums him up.

Hermann made his name a household word in the field of international dermatology, and honours were heaped upon him. He was, for example, Honorary Member of, among others, the American Dermatological Association (Vice-President, 1977), the American Academy of Dermatology (Vice-President, 1970), the Society for Investigative Dermatology (President, 1958), the American Society of Dermatopathology (President, 1964), and of numerous dermatological associations throughout the world, including the British Association of Dermatologists and the Scottish Dermatological Society. He received the Karl Herxheimer Award and Medal from the German Dermatological Society (1965), the Stephen Rothman Award and Medal from the Society for Investigative Dermatology (1974), the Lila Gruber Honor Award for cancer research from the American Academy of Dermatology (1978), and the First Founder's Award from the American Society of Dermatopathology (1984). He gave the von Zumbusch Memorial Lecture in Munich (1964) was the Dohi Memorial Lecturer in Japan (1967) and the Miles International Lecturer (1981). But, miraculously, he remained the same humble, kind and courteous man—a very rare feat. And we, who recognize his special quality, feel grateful for his life, and humble, in our turn, before his marvellous dedication and integrity.

Hermann Pinkus died in the presence of his wife on 18 May 1985 within a few weeks of what would have been their golden wedding anniversary, leaving Hilda and their son, Walter, to mourn. Everyone who knew Hermann and Hilda must pray that she shall be comforted in her loneliness.

A. LYELL

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# *Correspondence*

## Expression of T6 antigen on the epidermal keratinocytes in various dermatoses

MADAM, Intercellular epidermal staining for OKT6 has been described recently in skin biopsies from cutaneous B-cell lymphoma, lymphocytoma cutis and lymphocytic infiltration of the skin (Willemze, Dijkstra & Nugteren, 1985).

The occurrence of OKT6 positivity on keratinocytes was first described in a case of lichen sclerosus and atrophicus (Aiba & Tagami, 1984). In a comparative immunohistochemical study of the expression of HLA-DR antigen and T6-antigen in skin biopsies taken from patients with a variety of inflammatory skin disorders, we found expression of T6-antigen on keratinocytes in several cases (Drijkoningen *et al.*, 1986). In a recent study of skin biopsies from patients with the small plaque form of parapsoriasis, cutaneous T-cell lymphoma and cutaneous B-cell lymphoma, we again observed the presence of OKT6+ keratinocytes in several cases, whether or not a cell-free Grenz zone was present (Table 1). In a considerable number of these cases, HLA-DR+ keratinocytes were also present in serial sections. In all our cases, the occurrence of OKT6+ keratinocytes was accompanied by the occurrence of large numbers of OKT6+ Langerhans cells, as reported by Willemze *et al.* (1985).

No cytoplasmic staining of the keratinocytes for OKT6 was observed in any of the cases studied, rendering synthesis of the T6-antigen by keratinocytes unlikely. Thus, our findings also do not offer any explanation either for the occurrence of OKT6+ keratinocytes, or for their functional role.

TABLE 1. Occurrence of OKT6+ and HLA-DR+ keratinocytes in various dermatoses

| Skin disorder | Number of cases investigated | OKT6+ keratinocytes | HLA-DR+ keratinocytes | OKT6+ and HLA-DR+ keratinocytes |
|---|---|---|---|---|
| Dermatitis herpetiformis | 6 | 1 | 0 | 0 |
| Bullous pemphigoid | 11 | 1 | 0 | 0 |
| Fixed drug eruption | 1 | 1 | 0 | 0 |
| Pemphigus vulgaris | 6 | 2 | 0 | 0 |
| Contact dermatitis | 2 | 1 | 2 | 1 |
| Granuloma annulare | 1 | 1 | 0 | 0 |
| Malignant melanoma | 3 | 3 | 0 | 0 |
| Herpes gestationis | 1 | 1 | 0 | 0 |
| Graft-*vs*-host reaction | 1 | 1 | 1 | 1 |
| Culicosis bullosa | 1 | 1 | 1 | 1 |
| Acne varioliformis | 1 | 1 | 1 | 1 |
| Discoid lupus erythematosus | 6 | 1 | 3 | 1 |
| Nonpathognomic lesions | 2 | 1 | 0 | 0 |
| Small plaque parapsoriasis | 13 | 4 | 5 | 2 |
| Cutaneous T-cell lymphoma | 12 | 3 | 6 | 2 |
| Cutaneous B-cell lymphoma | 7 | 1 | 2 | 0 |

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Willemze, R., Dijkstra, A. & Nugteren, W.M. (1985) Intercellular distribution of HLA-DR and T6 antigens in the epidermis of various dermatoses. *British Journal of Dermatology*, **113**, 802.

Madam, In a recent letter, Willemze, Dijkstra and Nugteren (*British Journal of Dermatology*, 1985, **113**, 802) reported their findings regarding the distribution of intercellular T6 antigen in the epidermis of various dermatoses. In particular, they studied the relationship between the staining pattern with anti HLA-DR and anti OKT6 antisera in dermatoses characterized by a predominant T- or B-cell dermal infiltrate. Their results would indicate that the dermatoses in which a B-cell infiltrate without epidermotropism is observed show intercellular staining in the epidermis for OKT6 but not for HLA-DR, whereas the diseases characterized by a T-cell infiltrate, which tends to invade the epidermis, show an opposite pattern.

We have evaluated the presence of intercellular staining with OKT6 (Ortho Diagnostic) and HLA-DR (Becton Dickinson) mouse monoclonal antibodies in the epidermis of patients with various dermatoses using an immunoperoxidase technique with the avidin-biotin system (Su-Ming Hsu, Raine & Fanger, 1981). Both antisera were used at a dilution of 1:30. The results are summarized in Table 1.

One case of cutaneous B-cell lymphoma (CBCL), without any evidence of epidermotropism, showed both OKT6 and HLA-DR positive intercellular staining in the epidermis. In contrast, we observed a similar pattern in one case of mycosis fungoides (Fig. 1), one case of Sézary syndrome and one case of large plaque parapsoriasis.

TABLE 1. Intercellular staining for OKT6 and HLA-DR in the epidermis of various dermatoses

| Diseases | Total no. of cases | No. of cases showing epidermal intercellular staining: HLA-DR | OKT6 | Langerhans cells* |
|---|---|---|---|---|
| Cutaneous B-cell lymphoma | 3 | 1 | 1 | N |
| Mycosis fungoides | 5 | 5 | 1 | INC |
| Sézary syndrome | 3 | 3 | 1 | INC |
| Large plaque parapsoriasis | 2 | 2 | 1 | INC |
| Sarcoidosis | 1 | 1 | 1 | INC |
| Progressive systemic sclerosis | 3 | 0 | 1 | INC |
| Discoid lupus erythematosus | 3 | 2 | 2 | N |
| Pseudopelade of Brocq | 7 | 1 | 0 | N |
| Pityriasis lichenoides acuta | 4 | 4 | 0 | INC |
| Lymphomatoid papulosis | 1 | 1 | 0 | INC |

* N = normal numbers; INC = increased numbers.

Furthermore, we found the same OKT6 epidermal staining pattern in dermatoses other than lymphomas, including progressive systemic sclerosis, discoid lupus erythematosus (DLE) and sarcoidosis. In the latter two conditions, intercellular HLA-DR staining in the epidermis was also obtained. Aiba and Tagami (1984) observed a similar OKT6 epidermal staining in a case of lichen sclerosus et atrophicus.

We can confirm, in agreement with Willemze *et al.*, that in all our cases the intercellular epidermal OKT6 staining was generally weaker than that for HLA-DR antigen. The presence of intercellular OKT6 activity was associated with an increased number of epidermal Langerhans cells in all our patients, except for the cases of CBCL and DLE.

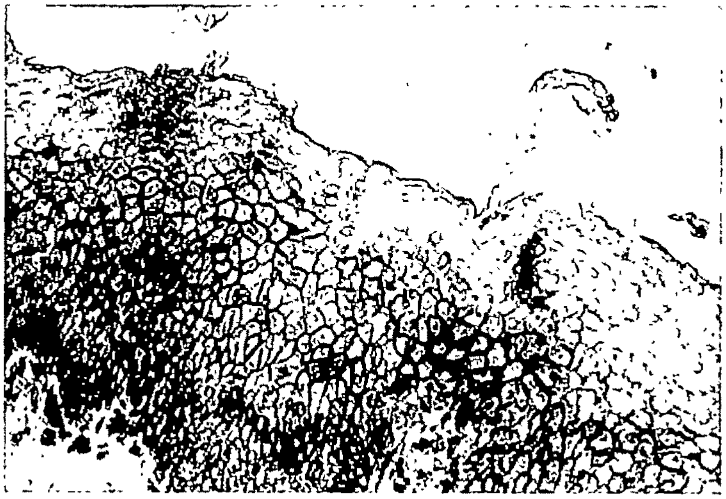

FIGURE 1. Mycosis fungoides. Staining with OKT6 antiserum, showing positive intercellular staining in the epidermis.

Our data indicate that this unusual intercellular OKT6 staining pattern is not a feature of one particular group of dermatoses, so it is difficult to support any hypothesis concerning the expression of T6 antigen by keratinocytes.

We believe that future studies on the function and properties of keratinocytes, together with immuno-electron-microscopy, will help to determine not only the exact localization of the T6 antigen but also its significance.

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## Reply

MADAM, In contrast to our study in which an intercellular epidermal staining with OKT6 antiserum was found only in non-epidermotropic conditions such as cutaneous B-cell lymphoma, lymphocytoma cutis and lymphocytic infiltration of the skin, two other groups now describe a similar staining pattern in a large variey of inflammatory and neoplastic skin diseases. In our study, only sections showing an intercellular distribution of T6 antigens over at least 25% of the interfollicular epidermis were considered positive. Although the present studies do not mention the criteria which have been used, it is not unlikely that they have also included cases with a focal intercellular OKT6 activity. Small groups of 'OKT6+ keratinocytes' adjacent to clusters of OKT6+ Langerhans cells are found regularly in various dermatoses, and also in follicular epithelium of normal skin. Whether such differences in selection criteria, and subsequent differences in results, really matter, may be questioned. There is general agreement that the presence of T6 antigens on keratinocyte membranes is associated invariably with highly increased numbers of OKT6+ Langerhans cells. In contrast, the presence of HLA-DR+ keratinocytes is often not parallelled by increased numbers of HLA-DR+ dendritic cells. In our recent study, it was noted that in particular in cases of lymphocytoma cutis, epidermal Langerhans cells were not only numerous, but also often had extremely long cytoplasmic extensions. For these reasons, we are committed to the view that passive absorption of excess Langerhans cell-derived T6 antigens by keratinocytes is the most likely explanation for this unusual intercellular staining pattern, and doubt whether any specific significance can be attributed to this phenomenon.

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## Natural killer cells and lymphomatoid granulomatosis

MADAM, After the discussion on the possible presence of high concentrations of natural killer (NK) cells in the skin infiltrate of lymphomatoid granulomatosis (Bos *et al.*, 1983, 1984; Rooney *et al.*, 1984) we report a patient with this disease.

A 66-year-old woman presented with a 4-month history of a painful indurated plaque on her left thigh, low fever and paresthesia of the legs. On examination, there was a large violaceous indurated plaque on her left buttock and three smaller erythematous nodules on her left thigh. An angiocentric and angiodestructive vasculitis in a granulomatous infiltrate with numerous atypical lymphoreticular cells was seen on biopsy.

A bilateral interstitial fibrosis was seen on X-ray of the chest and the patient therefore had a lung biopsy which disclosed an interstitial granulomatous angiocentric and angiodestructive vasculitis with atypical mononuclear cells.

Laboratory tests revealed a normal blood count, elevated ESR (50 mm/h), alkaline phosphatase (111 U/l; normal 30–85 U/l) and gamma GT (82 U/l; normal 0–65 U/l). Other tests revealed high levels of $\alpha 2$ and $\beta$ globulins, serum IgA (46·2 mg/l; normal 9·0–40·0 mg/l) and IgE (167 U/ml; normal less than 100 U/ml). ASO titre was high (400 U/ml; normal less than 200 U/ml). Urinalysis and creatinine clearance were normal. Circulating immune complexes (conglutinin method) were elevated (59%; normal 0–17%), but complement levels were normal. Rheumatoid factor, cold agglutinins and antinuclear antibodies were absent. HBsAg was absent, but anti-HBs and anti-HBc were present. Titres of antibodies to cytomegalovirus and Epstein-Barr virus were within the normal range. The T4/T8 ratio in the peripheral blood and in the bronchoalveolar lavage was normal (2·5:1).

Sinus X-rays were normal, but an abdominal sonogram revealed hepatomegaly with normal structure of the liver and biliary tree, and the presence of a solid, 1 cm diameter, nodule in the spleen. No lymphadenopathy was noted. EEG was normal, but computerized axial tomography with arteriography of the brain disclosed some small opacities along the vessels.

Direct immunofluorescence examination of a recent nodule showed C3 granular deposits in the basement membrane and in the vessels of the superficial and deep dermis.

Phenotypic characterization of the skin infiltrate indicated that most cells were mature T lymphocytes OKT11+ OKT3+ Leu9− OKT6− OKT9− and OKT10−. OKT4+ cells predominated in the centre of the infiltrate, whereas at its periphery only OKT8+ cells were present. Leu 11+ cells were rare (1–2%), while nearly 35% of the cells were Leu 7+. Double immunostaining of tissue sections was performed using anti-Leu 2a-phycoerythrin (PE)/anti Leu 7+ rat anti-mouse IgM FITC and anti-Leu 3aPE/anti-Leu 7+ rat anti-mouse IgM FITC. This showed that the majority of Leu 7+ cells (75%) were Leu 2+. In addition, some Leu 7+ Leu 3+ cells (20%) were found. The general OKT4/OKT8 ratio in the infiltate was 3:1.

A diagnosis of lymphomatoid granulomatosis was made and treatment with prednisone (1 mg/kg on alternate days) and cyclophosphamide (2 mg/kg/day, 4 g total dose) was started.

The patient died 9 months after the onset of skin lesions with high fever, cyclophosphamide-unrelated pancytopenia, multiple bilateral nodules in the lungs and a right hemiparesis.

Our case differs from that described by Bos *et al.*, as our patient had Leu7+ cells which were not mature NK cells but simply OKT8+ and OKT4+ lymphocytes co-expressing the Leu7 antigen. OKT8+ and OKT4+ cells co-expressing markers that define cells of NK lineage have been found in large numbers in chronic lymphocytic leukaemia (CLL) and regarded as reflecting an increase of immature NK cells. The high number of Leu7+ OKT3+ OKT8+ cells confirms the heterogeneity of the Leu 7+ subpopulation. Whether in lymphomatoid granulomatosis they are of prognostic significance with respect to the evolution into a frank lymphoma, as seems to be the case in B-CLL (Velardi *et al.*, 1985), remains obscure.

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## Persistent phototoxicity after benoxaprofen

MADAM, I was interested in the letter from Drs Mayou and Black (*British Journal of Dermatology*, 1986, **114**, 519) describing pseudoporphyria due to naproxen. It appears that the non-steroidal anti-inflammatory drugs produce a variety of light reactions.

There is now ample evidence that benoxaprofen, which was withdrawn in 1982 because of its side-effects, produces phototoxicity. The incidence of this side-effect was found to be as high as 50% in the U.K. in summer (Halsey & Cardoe, 1982). The resemblance of the intense burning and pain suffered by patients on benoxaprofen and exposed to summer sunlight to the symptoms of erythropoietic protoporphyria has been commented on by a number of authors, including Ferguson *et al.* (1982). There has been no blistering to suggest porphyria or pseudoporphyria.

Since it was assumed that the reaction was a phototoxic effect, it was believed that this reaction would cease within a short time if the drug was discontinued. I have had the opportunity of examining over 30 patients who suffered phototoxicity when exposed to sunlight whilst on benoxaprofen. Many stated that it occurred when sunlight had penetrated window glass or car windows, and a few also complained of symptoms in winter sunshine, which implies that UVA as well as or rather than UVB might be responsible.

In the description of their symptoms, the intensity of the pain was more severe than any other drug-induced phototoxicity that I have encountered. It is of interest that, in experimental work, Kligman and Kaidbey (1982) found that four out of five volunteers refused to continue light patch tests because of the intensity of the pain produced.

Of the 30 patients, 20 gave the classical history of burning like bee stings within 5–10 min of exposure to sunlight. Many of the patients had previously tanned normally and some had served in India. When the symptoms had come on they had had to run indoors and take a cold shower in order to relieve the discomfort. Although some described redness of the skin, many did not have a visible skin rash despite the intensity of the symptoms. Of the 20 patients who suffered from this type of phototoxicity, 10 have continued to feel burning of the skin in subsequent summers. The symptoms have occurred mainly without a visible rash, although some have had erythema. The effect has tended to become less severe each year, but many are frightened to take holidays in sunny parts of the world. Clothing and sunblocking creams have not given wholly satisfactory protection.

The only reference I have found to persistent phototoxicity after benoxaprofen has been a reference by Professor Inman of the Drug Surveillance Research Unit at the University of Southampton (1985). In his comment he does not appear fully convinced that benoxaprofen is responsible.

There are some resemblances in these patients to the effect of amiodarone where phototoxicity has persisted for as long as 4 months (Ferguson *et al.*, 1985).

I would be interested to hear whether others have encountered examples of persistent phototoxicity after benoxaprofen and of any suggestions as to the mechanism responsible.

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## Iontophoresis

MADAM, it is suggested by Dr Midtgaard (*British Journal of Dermatology*, 1986, **114**, 485) that treatment by iontophoresis 'has not been generally accepted . . . (partly because of the) . . . lack of suitable, readily available apparatus'.

Similar treatments for hyperhidrosis using glycopyrronium bromide have been given in this department for many years using the kind of direct current sources that are available in almost all physiotherapy departments. Abell and Morgan (1974) (quoted by Dr Midtgaard) state that they used d.c. apparatus 'widely available in physiotherapy departments'.

The new iontophoresis machine used by Dr Midtgaard apparently provides a direct current with an overlying pulsating direct current but what advantage this has, if any, over a simple direct current is not explained.

Iontophoresis using either tap water or an anticholinergic compound can be readily applied in most physiotherapy departments in this country.

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ABELL, E. & MORGAN, K. (1974) The treatment of idiopathic hyperhidrosis by glycopyrronium bromide and tap water iontophoresis. *British Journal of Dermatology*, **91**, 87.

## Arachidonic acid metabolism by polymorphonuclear leukocytes in psoriasis

MADAM, We read with interest the article by Maurice, Bather and Allen (*British Journal of Dermatology*, 1986, **114**, 57), who reported normal production of leukotriene $B_4$ ($LTB_4$), its isomers and 5-hydroxyeicosatetraenoic acid (5-HETE) from endogenous arachidonic acid after calcium ionophore A23187 stimulation of polymorphonuclear leukocytes in 19 patients with plaque psoriasis. Their data confirm our results (Ruzicka *et al.*, 1985), which were obtained in 19 patients with plaque, guttate and pustular psoriasis using a somewhat different method.

We measured $LTB_4$ and 5-HETE synthesis by polymorphonuclear leukocytes from exogenous radioactively labelled arachidonic acid, which allows the selective determination of 5-lipoxygenase activity, bypassing the phospholipase $A_2$ step. The assay described by Maurice *et al.* does not actually measure 5-lipoxygenase activity but rather the end products of the combined activities of phospholipase $A_2$, 5-lipoxygenase, epoxide hydrolase and $\omega$-hydroxylase.

Using our assay, we could not detect significant differences in $LTB_4$ and 5-HETE generating capacity between patients and controls, which is in agreement with the results of Maurice *et al.* In addition, $\omega$-hydroxylation products of $LTB_4$ were within the same range in patients and controls. However, our data do not exclude a role of 5-lipoxygenase metabolites in psoriasis, particularly in view of the report by Czarnetzki (1983), who showed an enhanced responsiveness to the action of $LTB_4$ in patients with psoriasis. Thus, even normal levels of $LTB_4$ might produce pathological effects in psoriasis patients. Receptor studies are being carried out in our laboratory which might provide new insights into the biological role of eicosanoids in psoriasis.

Finally, we would like to make a point about etretinate, since inexact citation or interpretation of drug data might lead to false deductions about the role of eicosanoids in psoriasis (Ruzicka, 1985a). The authors state that etretinate, which is used in psoriasis treatment, acts as a lipoxygenase inhibitor *in vitro*. They singled out work done with soybean lipoxygenase (Sircar & Schwender, 1983), an enzyme obviously not present in skin, but omitted reports dealing with retinoid effects upon cutaneous enzymes. Human cutaneous 12-lipoxygenase and polymorphonuclear leukocyte 5-lipoxygenase are not susceptible to inhibition by etretinate and other retinoids (Ruzicka, 1985b).

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## Reply

MADAM, We think Dr Ruzicka for his helpful comments on our study, the results of which were actually first published one year ago in the form of an abstract (Maurice, Allen & Bather, 1985).

We agree that in order to determine selectively the cellular activity of 5-lipoxygenase, the addition of exogenous substrate is useful because it bypasses the phospholipase step. However, the aim of our study was to assess the activity of the entire metabolic pathway, initiated by the release of free arachidonic acid from membrane phospholipids, and so we decided to incubate the cells with calcium ionophore alone. Moreover, one cannot assume that cells will handle exogenous substrate in the same way as substrate released endogenously. Because they have not separated out the isomers of leukotriene $B_4$ ($LTB_4$), the data of Ruzicka *et al.* does not exclude the possibility of an increased epoxide hydrolase activity in psoriatic polymorphs producing more $LTB_4$, but with an associated diminution in non-enzymic hydrolysis, so that the size of their '$LTB_4$' peak on HPLC remains the same. Our data does discount this possibility. We think, therefore, that in these respects the results of the two studies are complementary.

Although the data was not given in our paper, like Ruzicka *et al.* we found no difference in the formation of $\omega$-oxidation products between psoriatic and control cells, measured at 5 min. However, $\omega$-oxidation of $LTB_4$ by human polymorphs continues for longer periods (Powell, 1984), and in subsequent time-course experiments we have found some increase in the formation of $\omega$-oxidation products when measured at 20 min. In order to exclude definitively the possibility of an increase in both the synthesis and metabolism of $LTB_4$ (so that the levels at one point in time remain unchanged), it is necessary to measure the enzyme kinetics of the appropriate reactions and this investigation is currently in progress.

We certainly agree that our data does not exclude a role for 5-lipoxygenase products in psoriasis and there may indeed be increased production of $LTB_4$ by another cell type, such as the the keratinocyte. We would, however, in this context, question the relevance of the enhanced *in vitro* responsiveness of psoriatic monocytes towards $LTB_4$, reported by Czarnetzki (1983). We believe that it is unlikely that 'normal' levels of $LTB_4$ will produce pathological effects in psoriatic patients, in view of the decreased *in vivo* responsiveness of psoriatic skin to topical application of $LTB_4$ reported by Wong, Camp and Greaves (1985).

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# *Book Reviews*

**Papillomaviruses.** CIBA FOUNDATION SYMPOSIUM 120 (1986) Chichester: John Wiley. Pp. 259. Price £27.50.

This volume reports the CIBA Foundation Symposium held in July 1985. Immaculately presented, with an index which covers both the papers given and their discussion, it is as up-to-date as one can hope for where such a burgeoning topic is concerned. The participants were recognized experts from many countries, and the U.K. was well represented.

Of the 14 papers, a few are of little interest (or indeed comprehensibility) to the dermatologist. The others, however, contain much of clinical relevance; these deal with diagnosis, criteria for oncogenesis, papillomavirus infection in cattle and the possibility of immunization, epidermodysplasia verruciformis, HPV and carcinoma of the cervix, persistence and expression of HPV DNA in genital cancer, laryngeal papillomatosis and interferon. The discussion, whether as a result of good editing or cogent contributions or both, not only reads smoothly but reflects spoken speech, yet without the non-sequiturs so common in everyday life. Of many interesting papers, I would mention the final discussion session on epidemiological trends, legal aspects, immuno-suppression and sites and transmission of infection.

The book is redolent of a pleasing intellectual excitement and will be useful to many types of reader, certainly including the dermatologist.

C.M.RIDLEY

**Essentials of Industrial Dermatology.** W.A.D.GRIFFITHS and D.S.WILKINSON (1985) Oxford: Blackwell. Price £12.50.

This book, which can easily be read in a matter of a few days, covers all the important areas of industrial dermatology, including not only clinical, but also preventative, investigative and medicolegal aspects. It is primarily intended for factory medical officers with no specialized knowledge of dermatology and for those in dermatological training. As the book points out, however, many dermatologists in the U.K. have had little or no formal training in this very important aspect of dermatology and I am sure that many fully trained dermatologists will find it worthwhile reading.

There is a multi-author format, which does have some disadvantages and the book tends to be repetitive, but this is not such a bad thing, as many of the repeated lessons are most important. There is also some unevenness in the quality of the chapters, but in general, the standards are high. There are a few minor inaccuracies. It is to be hoped that future editions will distinguish the standard patch test reagent PPD from PPD mix (black rubber mix).

A feature of the book is the ability of most of the authors to impart successfully their considerable experience of the subject to the reader in a concise way. Many observations will be of great help to those engaged in the writing of medical reports. The book is inexpensive and I can strongly recommend it for the bookshelves of all practising dermatologists.

M.H.BECK

**Pinkus Guide to Dermatohistopathology.** 4th edition. AMIR MEHREGAN (1986) Norwalk, Connecticut: Appleton Century Crofts. ISBN 0-8385-7849-7. Pp. 655. Price $95.00.

Most dermatopathologists will open this fourth edition of Pinkus, which carries a charming picture of him as a frontispiece, with a feeling of considerable nostalgia. They should, however, be very much reassured that his friend and colleague Amir Mehregan has done him justice. This fourth edition continues the Pinkus tradition of being a dermatohistopathology text which stimulates thought, as well as offering guidance, while at the coal face of the microscope bench. An excellent chapter on immunofluorescence has been added and the book can be confidently recommended to all those with an established, or growing, interest in the important subject of dermatopathology.

RONA M.MACKIE

*British Journal of Dermatology* (1986) **115**, 520.

# *News and Notices*

## Skin Club Meetings 1986/1987

*27 November 1986 – London.*—'Epidermal Growth Factor'. Organiser: Dr D.Basketter.

*5 March 1987 – Greenford.*—'Markers of Epidermal Differentiation', joint meeting with Glaxo Laboratories. Organiser: Dr D.M.Turner.

*7 May 1987 – Glasgow.*—'Basement Membranes', joint meeting with Scottish Skin Biology Club.

*9 July 1987 – London.*—'Skin Equivalents'. Organiser: Dr I.Leigh.

For further details please contact: Dr P.J.Dykes, Department of Medicine (Dermatology), University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, U.K.

## The University of Liverpool—Mersey Regional Council for Postgraduate Medical Education—The Fourth 2-Day Course in Paediatric Dermatology

This course for Consultants, Senior Registrars and Registrars recently appointed in Dermatology, will be held on Thursday, 14 and Friday, 15 May 1987 and will be limited to 12 participants. Full details from: Dr J.L.Verbov, c/o Postgraduate Dean's Office, Faculty of Medicine, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K. Telephone: 051-709-3114.

## Relationship of Skin Colour and Skin Function

A one year postdoctoral fellowship is available to study the relationship of skin colour and skin function. The techniques will emphasize skin biology and skin bioengineering. For information, write to: Howard I.Maibach, M.D., University of California Medical School, Department of Dermatology, Box 0989, San Francisco, CA 94143-0989, U.S.A.

## ESDR Poster Awards

At the recent ESDR/SID Joint Meeting the winners of the two awards were:

*Foundation for Research—donated by Professor F.Serri*: 'Detection and localization of keratin mRNAs in mouse tongue epithelium by *in situ* hybridization with specific cDNA clones.'—Martin Rentrop, Bernhard Knapp, Jurgen Schweizer, Hermelita Winter, Institute of Experimental Pathology, German Cancer Research Center, Heidelberg, F.R.G.

*Friends of the Bill Reed Travel Award*: 'Placental-pemphigoid: a bullous pemphigoid-like dermatosis after injections of placental extracts in a man. Detection of antibodies to cutaneo-placental antigens.'—Catherine Fournier, Liliane Didierjean, Pierre Carraux, Denis Salomon, Yves Merot, Jean-Hilaire Saurat, Clinique de Dermatologie, Hôpital Cantonal, Universitaire de Geneve, 1211 Geneve 4, Switzerland.

## North of England Dermatological Society—Roy Summerly Memorial Prize

Following Roy Summerly's death in 1984, the North of England Dermatological Society launched a prize in his memory. This is intended to remember and salute Roy Summerly's contribution to dermatology, not least his clinical acumen which was widely sought and respected. The prize is presented to junior staff in training, for the best case presentation at North of England Dermatological Meetings. This year marks the first award and the Society is pleased to announce that Dr Marsden, Senior Registrar, Nottingham was the prize winner for his case presentation 'Pyoderma gangrenosum, subcorneal pustulosis and IgA paraproteinaemia' at the clinical meeting held in Nottingham on the 11 March 1986. The prize was awarded by Dr Keczkes, the President of the Society, at the Annual General Meeting held in Manchester on the 13 May 1986.

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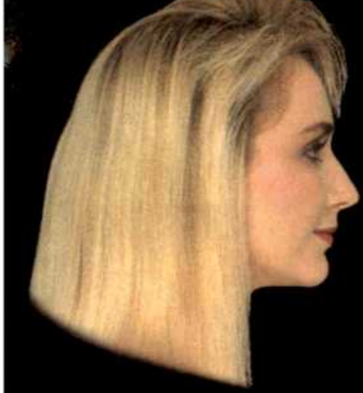

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tion 0036/5008. Application PC 0036/5005. ALPHOSYL HC. **Uses** Treatment of inflamed psoriasis. **Dosage & Administration** Apply 2 to 4 times daily. **Contra-indications, warnings etc.** Should not be used in skin
ections. Should not be used extensively in pregnancy. Avoid long-term use in infants. **Presentation** Contains refined alcoholic extract of coal tar 5%, allantoin 2%, hydrocortisone PhEur 0.5%. **Basic NHS cost** 30g
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|---|---|---|---|---|---|
| gram(s) | g | minute(s) | min | molar | mol/l |
| kilogram(s) | kg | centimetre(s) | cm | millilitre(s) | ml |
| milligram(s) ($10^{-3}$g) | mg | second(s) | s | gravitational acceleration | *g* |
| microgram(s) ($10^{-6}$g) | μg | cubic millimetre(s) | $mm^3$ | micrometre(s) | μm |
| nanogram(s) ($10^{-9}$g) | ng | millimetre(s) | mm | per cent | |
| picogram(s) ($10^{-12}$g) | pg | millicurie(s) | mCi | isotopic mass number placed as | [illegible] |
| hours(s) | h | milliequivalent | mmol | | |

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# British Journal of Dermatology, Volume 115, Number 4, October 1986

CONTENTS PAGE

**Invited Editorial**

Do epidermotropic T cells exist in normal skin? A re-evaluation of the SALT hypothesis 389
S.M.BREATHNACH

**Clinical and Laboratory Investigations**

Scl 70 antibody—a specific marker of systemic sclerosis 393
MARIA JARZABEK-CHORZELSKA, MARIA BLASZCZYK, STEFANIA JABLONSKA, T.CHORZELSKI, V.KUMAR AND E.H.BEUTNER

Degradation of porcine dermal connective tissue by collagenase and hyaluronidase 403
K.FRIEDMAN, S.V.POLLACK, T.MANNING AND S.R.PINNELL

Immunolocalization of collagenase and tissue inhibitor of metalloproteinases (TIMP) in hypertrophic scar tissue 409
ROSALIND M.HEMBRY AND H.P.EHRLICH

An open study of vitamin $D_3$ treatment in psoriasis vulgaris 421
S.MORIMOTO, K.YOSHIKAWA, T.KOZUKA, Y.KITANO, S.IMANAKA, K.FUKUO, E.KOH AND Y.KUMAHARA

Successful treatment of psoriasis with topical application of active vitamin $D_3$ analogue, $1\alpha$,24-dihydroxycholecalciferol 431
T.KATO, M.ROKUGO, T.TERUI AND H.TAGAMI

Circulating antibodies and antigenic cross-reactivity in *Hendersonula toruloidea* and *Scytalidium hyalinum* infections 435
M.K.MOORE AND R.J.HAY

HLA antigens and Langerhans cell density in contact dermatitis 447
S.I.WHITE, P.S.FRIEDMANN AND A.STRATTON

A quantitative study of the effect of topical indomethacin on cutaneous erythema induced by UVB and UVC radiation 453
P.M.FARR AND B.L.DIFFEY

Behavioural treatment of scratching in patients with atopic dermatitis 467
L.MELIN, T.FREDERIKSEN, P.NOREN AND B.G.SWEBILIUS

Effect of percutaneous absorption of hydrocortisone on adrenocortical responsiveness in infants with severe skin disease 475
M.TURPEINEN, O.P.SALO AND S.LEISTI

Challenge reactions in atopic dermatitis after percutaneous entry of mite antigen 485
A.GONDO, N.SAEKI AND Y.TOKUDA

**Case Reports**

Secondary syphilis mimicking Sweet's syndrome 495
H.F.JORDAAN AND J.CILLIERS

Giant orf in a normal individual 497
J.V.S.PETHER, C.J.W.GUERRIER, S.M.JONES, A.E.ADAM AND W.N.KINGSBURY

Blisters over burn scars in a child 501
R.PEDRAGOSA, S.SERRANO, J.CAROL-MURILLO, J.V.HERNANDEZ AND J.VIDAL

**Obituary**

Hermann Pinkus 507

**Correspondence**

Expression of T6 antigen on the epidermal keratinocytes in various dermatoses (I) 511
M.DRIJKONINGEN, C.DE WOLF-PEETERS AND V.DESMET

Expression of T6 antigen on the epidermal keratinocytes in various dermatoses (II) 512
C.PINCELLI, G.GIROLOMONI AND L.BENASSI

Reply 513
R.WILLEMZE

Natural killer cells and lymphomatoid granulomatosis 514
F.RONGIOLETTI, MARIA GRAZIA BERNENGO, F.CROVATO AND A.REBORA

Persistent phototoxicity after benoxaprofen 515
I.B.SNEDDON

Iontophoresis 516
J.LOW

Arachidonic acid metabolism by polymorphonuclear leukocytes in psoriasis 517
T.RUZICKA

Reply 518
P.D.L.MAURICE AND B.R.ALLEN

**Book Reviews** 519

**News and Notices** 520

# British Journal of Dermatology

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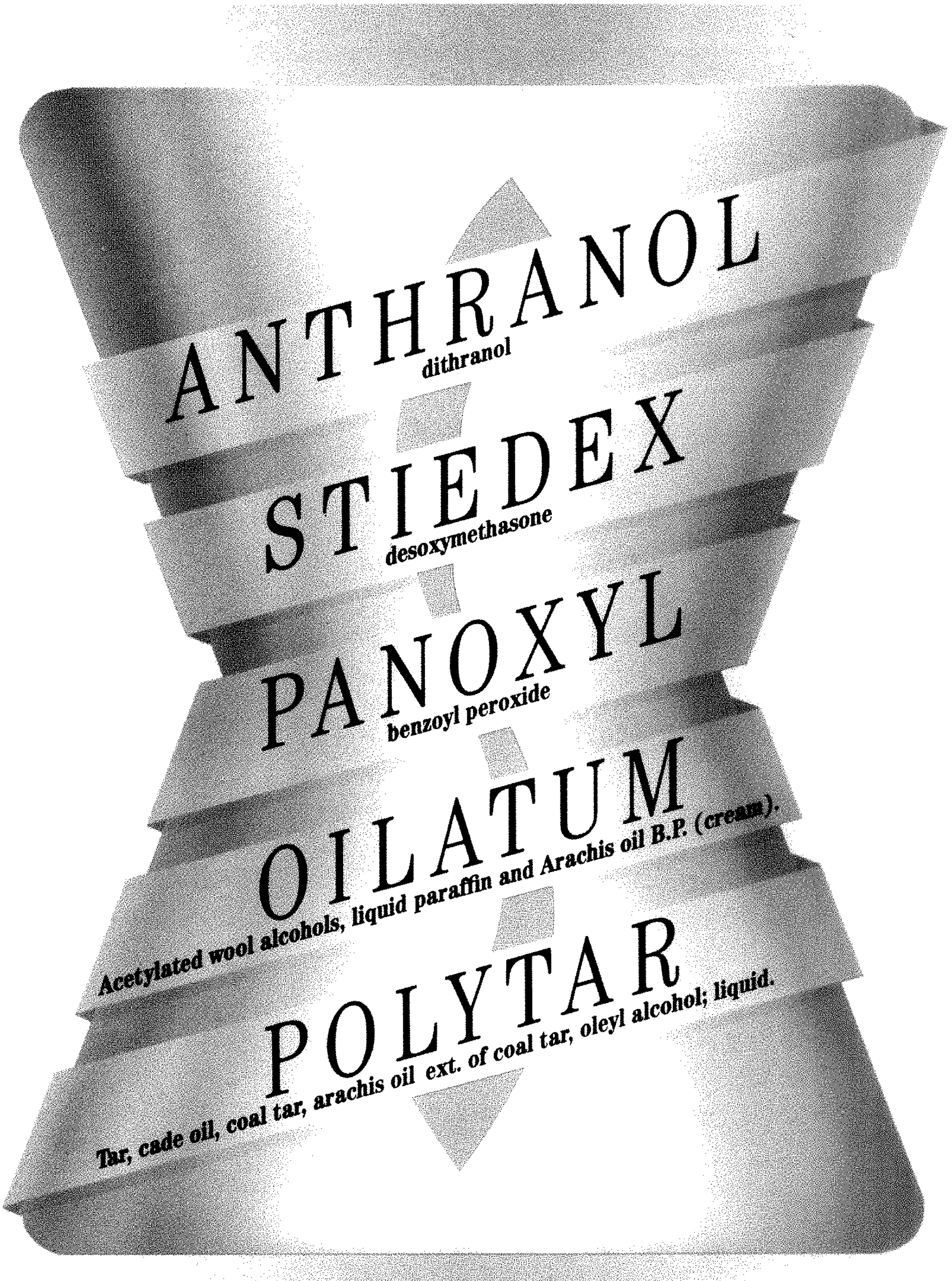
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0 19 261623 4, 117 pp., illus., November 1986 £10

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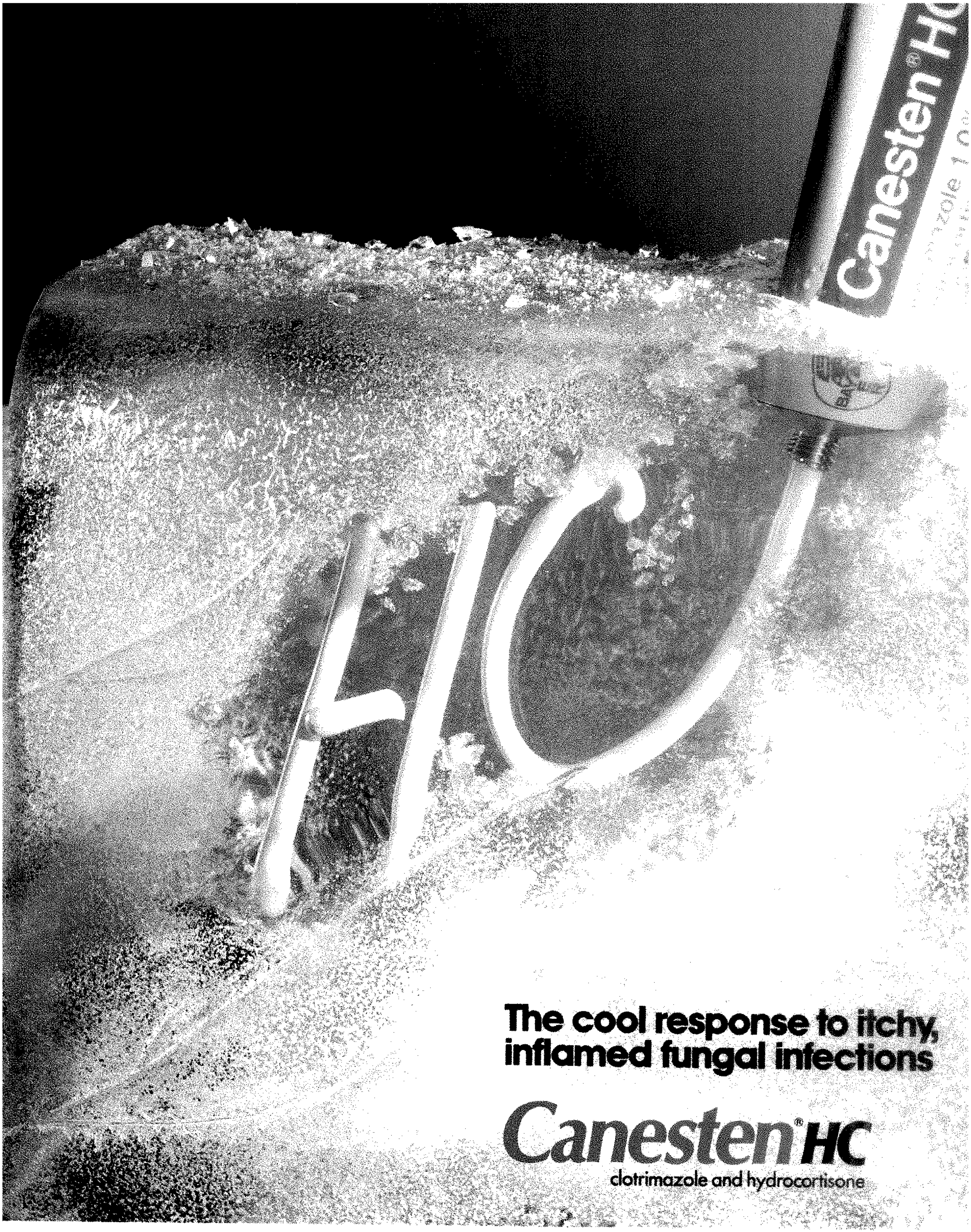
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*British Journal of Dermatology* (1986) **115**, 521–528.

# A relative thrombocytosis and elevated mean platelet volume are features of gravitational disease

M.J.D.GOODFIELD

Department of Dermatology, University Hospital, Nottingham, U.K.

Accepted for publication 10 June 1986

## SUMMARY

Platelet counts were obtained in 60 patients with gravitational ulceration (GU), 60 with gravitational eczema (GE), 56 with endogenous eczema (EE) and 60 normal controls. Mean platelet volume (MPV), a broad index of platelet size, was estimated in the last 40 patients in each group (29 with EE). Causes of a reactive thrombocytosis were excluded. Platelet counts were significantly higher in both gravitational groups than in the EE group or the normal controls ($P=0{\cdot}001$). Ulcer healing produced no significant changes in platelet count, and patients with non-gravitational ulcers had normal platelet counts.

MPV was significantly higher in both gravitational groups than in controls after correction for platelet count, by analysis of co-variance ($P=0{\cdot}01$).

These differences in platelet parameters in gravitational disease suggest a possible role for platelets in this disease, and platelet inhibiting therapy may be of value at an early stage in the development of the condition.

Chronically elevated peripheral venous pressure leads to the formation of abnormal capillaries in the skin of the lower leg (Browse, 1983). The presence of a fibrin barrier to the diffusion of oxygen and nutrients from these capillaries is said to be the cause of venous ulceration (Burnand *et al.*, 1982). There are, however, patients with long-standing venous disease who never develop cutaneous signs of gravitational disease, and there must therefore be additional factors determining those individuals who do progress to eczema and frank ulceration. Thrombocytosis is a common finding in patients with venous ulceration and is usually said to be a secondary event, although there is little evidence for this claim. Thrombocytosis, primary or secondary in aetiology, is associated with thrombotic events (Morowitz, Allen & Kirsner, 1968), and so it is important that the nature and cause of gravitational thrombocytosis is more fully documented.

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With the advent of automated platelet counting, further indices of the platelet population are now available, allowing better documentation of abnormalities in platelet behaviour. One such index is the mean platelet volume (MPV). Automated platelet counters, such as the Coulter Type S, directly count particles of different sizes, construct a histogram of the numbers in each size division and fit a log normal curve to this distribution (Rowan, 1983). Total platelet count and MPV are calculated from the curve. It has been shown that MPV falls with increasing platelet count up to very high counts (Giles, 1981), and that larger platelets are functionally more active (Mannucci & Sharp, 1967). The pattern of change with platelet count will help further to define the thrombocytosis as primary or secondary; any absolute increase in this parameter would suggest a more fundamental platelet involvement in the aetiology of gravitational disease.

The aims of the present study were threefold: firstly, to confirm the relative thrombocytosis of all stages of gravitational disease; secondly, to attempt to determine whether this is a primary event, and thirdly, to investigate the MPV as an additional parameter of platelet morphology and as a further indicator of their pathogenic role.

## METHODS

Five groups of patients attending the out-patient department were included in the study. They were broadly matched for age and sex. They were divided according to diagnosis:

*Group 1*. (60 patients). Gravitational ulceration, characterized by ulcers occurring in typical sites on the lower leg in patients with pigmentary and/or inflammatory changes of venous disease.

*Group 2*. (60 patients). Gravitational eczema, with signs of venous disease, but with controlled eczema.

*Group 3*. (56 patients). Endogenous eczema, with controlled disease matched for severity with Group 2.

*Group 4*. (60 patients). Normal controls, with no signs of venous disease, presenting with benign skin tumours.

*Group 5*. (20 patients). Patients with non-gravitational leg ulcers.

Patients with a history or evidence of gastro-intestinal disease, haematological disorder, inflammatory conditions known to influence platelet count, and those taking drugs known to influence platelet behaviour, or patients with active infection of the ulcer site, were excluded.

A full blood count, platelet count, ESR and defibrinated ESR and C-reactive protein, iron studies and appropriate bacteriology were performed in all patients. Further investigations, to define any abnormalities, were performed as indicated. MPV was measured in the last 40 patients in Groups 1, 2 and 4 and in the last 29 in Group 3.

Statistical analysis of the results was by analysis of variance, with Scheffe's technique for multiple comparisons, and by linear regression, with comparison of slopes of the regression lines of the study groups.

TABLE 1. Age and sex of patients and controls

| Group | | Sex | | Age (years) | |
|---|---|---|---|---|---|
| | | M | F | Mean | Range |
| 1. | Gravitational ulceration | 16 | 44 | 74 | 52–91 |
| 2. | Gravitational eczema | 20 | 40 | 68 | 28–92 |
| 3. | Endogenous eczema | 23 | 33 | 64 | 40–80 |
| 4. | Normal controls | 21 | 39 | 74 | 42–91 |
| 5. | Non-gravitational ulcers | 9 | 11 | 52 | 20–70 |

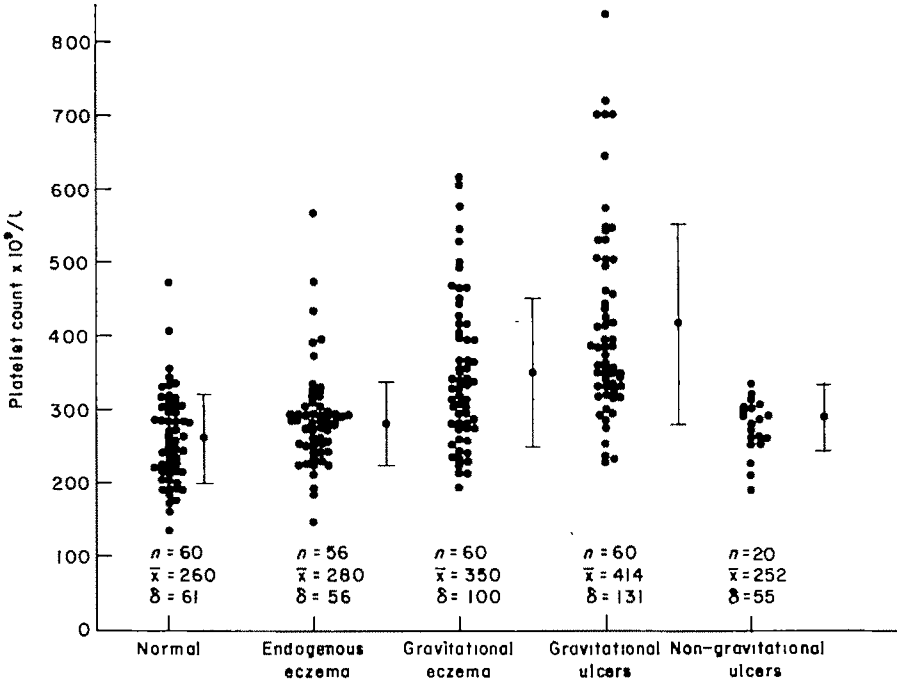

FIGURE 1 Platelet counts by group ($n$ = number; x̄ = mean; $\delta$ = standard deviation). Bars indicate standard deviation.

## RESULTS

The age range and sex ratio in the groups of patients studied are shown in Table 1, and indicate broad similarity between the groups.

Figure 1 shows platelet counts in the five groups studied. An analysis of variance showed highly significant differences in platelet counts between the groups (*F* ratio 29·5, *F* probability 0·001), and the Scheffe method showed that these differences (at an $\alpha$ of 0·01) occurred between

TABLE 2. Platelet counts in patient and control groups

| | Platelet count × $10^9$/l | | | | |
|---|---|---|---|---|---|
| | Normal controls | Non-gravitational eczema | Endogenous eczema | Gravitational eczema | Gravitational ulceration |
| Mean ± SE | 259 ± 12·6 | 290 ± 21·8 | 288 ± 14·1 | 351 ± 12·6 | 414 ± 12·6 |

Underlining indicates those groups which do not differ significantly from each other ($\alpha = 0{\cdot}01$).

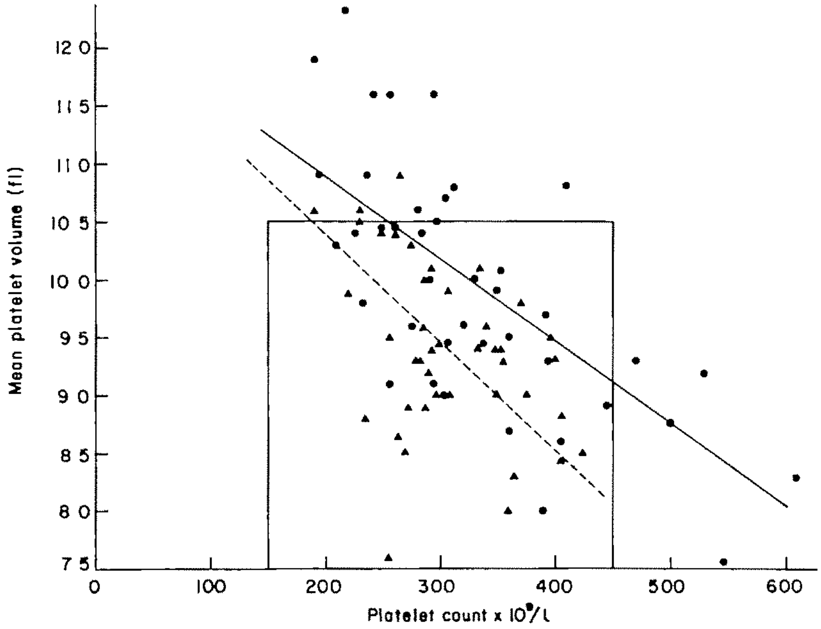

FIGURE 2. ▲ Controls; $r = 0{\cdot}594$; $P < 0{\cdot}01$. ● Gravitational eczema; $r = 0{\cdot}65$; $P < 0{\cdot}01$. Box indicates accepted normal values.

the two gravitational groups and the three other groups. These differences are summarized in Table 2, which also indicates the standard error of the differences. There was no difference between the two gravitational groups at this level of significance. There was also no significant change in platelet count on ulcer healing (paired *t*-test, $P > 0{\cdot}1$).

The relative thrombocytosis in both gravitational groups was not explained by any of the accepted causes of a reactive process, acute blood loss, iron deficiency, chronic infection or acute inflammation, e.g. rheumatoid arthritis or ulcerative colitis, which were all excluded by investigation. In particular, and most importantly, the abnormality was not shared by the smaller group of patients with non-gravitational ulcers of mixed aetiology, nor by patients with another inflammatory dermatosis—endogenous eczema.

The known relationship between MPV and platelet count was confirmed for all groups. As an example, Figure 2 shows results for normal controls and the gravitational eczema group, and suggests a quantitatively different relationship between MPV and platelet count in the disease group, although the difference in the slopes of the regression lines was not statistically significant ($P = 0{\cdot}1$).

Figure 3 indicates the MPV in each group. There were small differences in MPV, with higher values in the gravitational groups. An analysis of variance, with platelet count as a co-variate to correct for the marked differences in counts between groups, revealed highly significant increases in MPV in the gravitational groups ($F$ ratio 11·62; $P < 0{\cdot}001$). The Scheffe procedure showed these to be between the gravitational groups and the other two groups which were not significantly different from each other (at an $\alpha$ of 0·01). These results are summarized in Table 3.

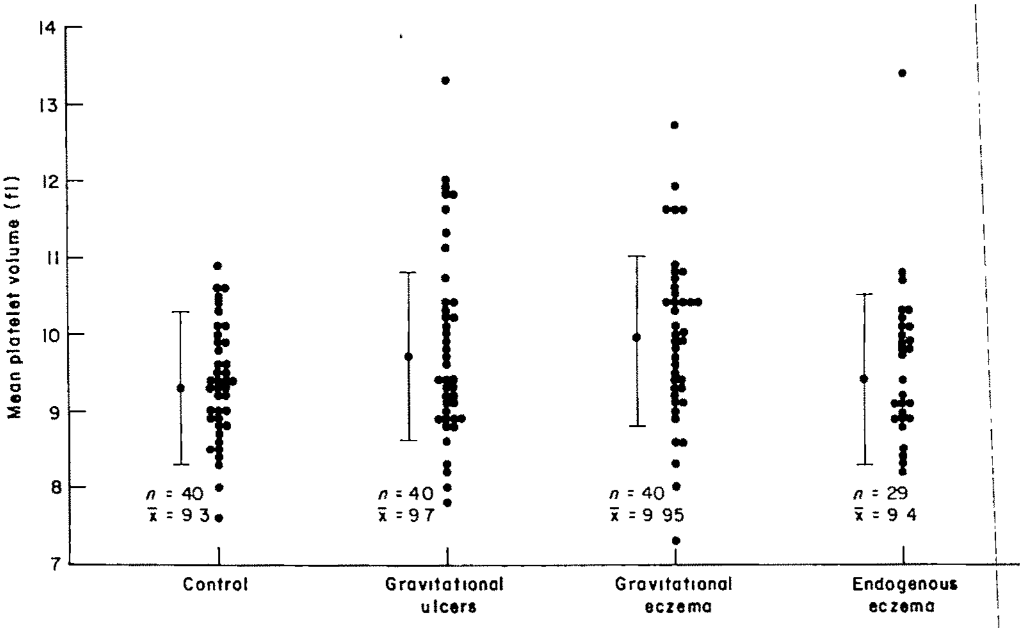

FIGURE 3. Mean platelet volume by group. Bars indicate standard deviation.

The platelet-crit, a combined measure of count and volume, indicating the platelet mass, was significantly elevated in the gravitational groups ($F = 28{\cdot}4$; $P < 0{\cdot}001$) (Fig. 4).

TABLE 3. Mean platelet volume (MPV) in patient and control groups

| | MPV (fl) | | | |
|---|---|---|---|---|
| | Normal controls | Endogenous eczema | Gravitational eczema | Gravitational ulceration |
| Mean ± SE | 9·30±0·01 | 9·40±0·02 | 9·95±0·01 | 9·70±0·01 |

Underlining indicates those groups which did not differ significantly from each other (α = 0·01). The MPV means were adjusted to correspond to a platelet count of 300 in all groups.

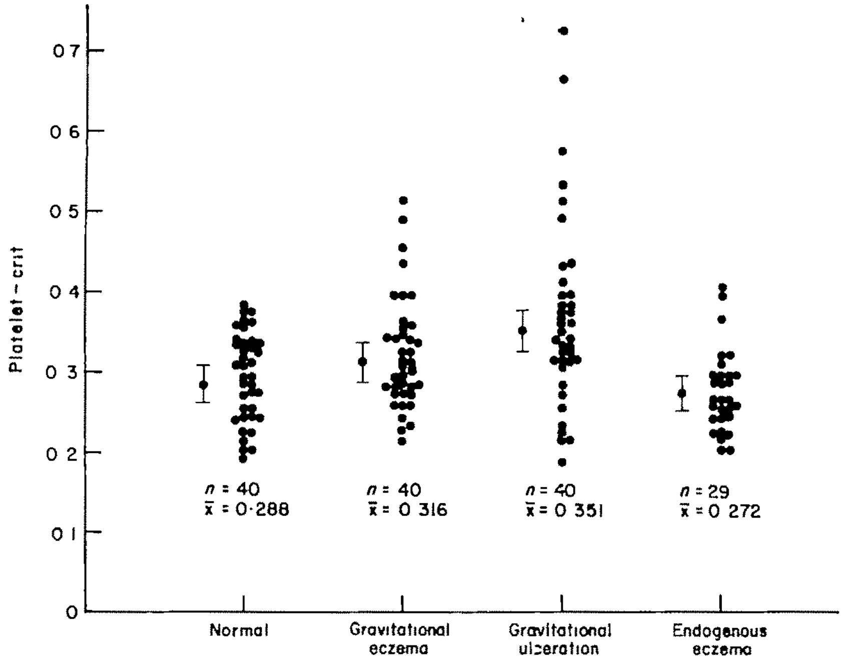

FIGURE 4. Platelet count by group. Bars indicate standard deviation.

## DISCUSSION

Elevated peripheral venous pressure, whether due to congenital or acquired venous disease, or as a result of artificial A-V fistulae (Wood, Reilly & Smith, 1983), produces abnormal capillaries which leak fibrinogen, and are surrounded by a relatively impermeable fibrin barrier. Affected limbs have lower levels of fibrinolysis (Wolfe, Morland & Browse, 1979), and the beneficial effects of stanazolol, a fibrinolytic agent, have been described (Burnand *et al.*, 1980). It has been suggested that platelet thrombi are implicated in the aetiology of atrophie blanche, and that anti-platelet agents improve this condition (Milstone *et al.*, 1983). Platelets contain fibrinogen (Gokcen & Yumis, 1963), and its presence is essential for some platelet functions (Mustard *et al.*, 1978). It is therefore possible that platelet behaviour is of significance in the aetiology of gravitational disease, and changes in platelet number and size may indicate this involvement.

The relative thrombocytosis confirmed in the present study is common to all stages of gravitational disease examined, and seems to be specific to this sort of cutaneous inflammation. It is not a feature of other types of controlled cutaneous inflammation. The common causes of a reactive thrombocytosis were effectively excluded. Although anaemia is a common finding in patients with frank ulceration, this is not due to iron deficiency, and is not a feature of patients with venous eczema. The ESR is uniformly high in gravitational ulceration, but defibrinates to normal levels, indicating high levels of fibrinogen. The ESR is normal in patients with gravitational eczema, but more direct measures of fibrinogen would be of value in view of the role of this protein in platelet function and venous disease. Patients with active infection of the ulcer site were excluded from the study, both by clinical assessment, bacteriology, and by the presence of a raised white blood count or CRP level. This finding therefore, seems to be a primary feature of gravitational disease. It is possible that it represents a quantitatively different response to inflammation, but this seems unlikely, particularly in view of the associated changes in MPV. These add further weight to the argument that platelets may be of more fundamental importance in this condition than is currently believed. The normal inverse relationship between count and volume still holds, but, at all levels of platelet count, MPV was higher in the patients with gravitational disease.

The broad index, the MPV, does not indicate whether there is a lack of small platelets or an excess of larger, theoretically more active, platelets as a primary abnormality. This is an important consideration, and needs to be established. There have been suggestions that a raised MPV is associated with a condition in which vascular occlusion may be of importance (Cameron *et al.*, 1983), but not in association with the compounding abnormality of a raised platelet count. The combination could arise as a consequence of a low grade platelet consumption in the abnormal vessels of the lower leg due to endothelial damage, but this finding in itself would be of some significance. It is also possible that the two abnormalities do not occur together, but that each is independently associated with gravitational disease. The platelet mass, indicated by the platelet-crit, would then be the most valuable index of risk for the disease.

There is sufficient evidence to merit further investigation of platelet behaviour in this clinical context, aimed at confirming this as a primary event in venous disease. There is also the hope that manipulation of platelet behaviour, with a number of more or less specific agents, might allow intervention at an early stage in this debilitating condition. Further confirmation and definition are essential, but it seems likely that a relative thrombocytosis and elevated MPV are indeed important features of gravitational disease.

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# Support and stimulation of epidermal cell outgrowth from porcine skin explants by platelet factors

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## SUMMARY

The effect of a platelet homogenate fraction (PHF) on epidermal cell outgrowth from porcine skin explants was examined in culture medium with and without 5% foetal bovine serum (FBS). Explants were sustained and outgrowth was initiated, supported and stimulated by PHF, in the absence of FBS. PHF-supported outgrowth was concentration-dependent up to 130 μg protein/ml. PHF activity was not removed by heat treatment at 100°C for 2 min but was destroyed by heating at 100°C for 5 min. The factor responsible for epidermal cell outgrowth in PHF may be epibolin or serum spreading factor, or may be a platelet-derived factor as yet undescribed. Viability and outgrowth were stimulated by the 'high molecular weight' fraction of PHF ($>30$ kd). PHF plus 5% FBS gave inhibition of outgrowth, which was not PHF concentration-dependent; however, this inhibitory effect was variable with different PHF preparations. This study is the first to demonstrate an effect of platelet-derived factors on epidermal cell viability and outgrowth.

The first responses to skin injury are bleeding and coagulation. Blood enters the wound, a clot is formed by activated platelets and fibrin, and specific blood components, including platelet-derived factors, are released at the site of injury. Blood-derived growth factors were first proposed by Balk (1971), who demonstrated that normal chick embryo fibroblasts grow well in serum-supplemented medium, but not in plasma-supplemented medium. Ross *et al.* (1974) and Kohler and Lipton (1974) showed that platelets are the source of most of the mitogenic activity of serum required by normal, non-transformed smooth muscle cells and fibroblasts. Their studies led to the hypothesis that platelets activated at the site of vascular injury release

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Portions of this work were presented at the 1983 and 1984 Annual Meetings of the Society for Investigative Dermatology (Hebda & Eaglstein, 1983, 1984).

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components that trigger proliferation (Ross *et al.*, 1974; Harker *et al.*, 1976). Studies based on this hypothesis resulted in the isolation and characterization of platelet-derived growth factor (PDGF) (Antoniades, Scher & Stiles, 1979; Ross & Vogel, 1978), which is a potent mitogen and chemoattractant for smooth muscle cells (Ross *et al.*, 1974; Grotendorst *et al.*, 1981) and fibroblasts (Kohler & Lipton, 1974; Seppa *et al.*, 1982), both key participants in dermal wound healing. Experimental evidence suggests that PDGF is specific for mesenchymal cells. Heldin, Westermark and Wasteson (1981) found that receptors for PDGF are restricted to connective tissue and glial cells. In addition to PDGF, platelets have been shown to contain other growth factors; however, the rested target cells have been primarily mesenchymal cells (for review see Westermark *et al.*, 1983). Also, Kondo and Amano (1980) reported that when a factor released from platelets was added to plasma-supplemented medium, there was an increase in the size and number of keratinocyte colonies compared with control keratinocyte cultures grown in medium with platelet-poor plasma. Eastment and Sirbasku (1980) demonstrated that platelet extracts were mitogenic for an epithelial tumour cell line (MTW9/PL); but the activity was separable from PDGF. These two studies suggest the possibility that platelets contain factors mitogenic for epidermal cells.

Epidermal cell outgrowth from skin explants (Hammar & Halprin, 1981) is a good *in vitro* model for re-epithelialization because the tissue structure of the epidermis and dermis is partially retained. Although systemic responses are absent in this model, localized interactions, including dermal-epidermal and basement membrane-epidermal interactions, may occur. Outgrowth from explants requires attachment, spreading and migration of epidermal cells from the edges of the explant. As the epidermal sheet grows out, cells begin to divide and then to stratify and mature (Hammar & Halprin, 1981). Thus, many of the cellular events involved in epidermal cell outgrowth from explants are similar to re-epithelialization events *in vivo*. The explant culture system has been utilized to evaluate potential effectors of epidermis, including hormones, growth factors and inhibitors (Flaxman & Harper, 1975; Fischer *et al.*, 1980; Taylor *et al.*, 1983). In the present study, an interest in epidermal healing prompted us to determine the effects on skin explants of a platelet homogenate and a commercially available PDGF preparation, in order to investigate the possibility that platelets contain factors that influence epidermal cell activity.

## METHODS

RPMI 1640 medium, foetal bovine serum (FBS) and antibiotic/antimycotic (AB/AM) (penicillin 10 000 units/ml, streptomycin 10 000 μg/ml, fungizone 25 μg/ml, after rehydration in 20 ml water) were obtained from GIBCO (Grand Island, NY, U.S.A.). Commercial platelet-derived growth factor (PDGF) was purchased from Bethesda Research Labs (Bethesda, MD, U.S.A.). Human platelets were obtained from the Central Blood Bank (Pittsburgh, PA, U.S.A.). The platelets were not more than one week old and were physiologically normal.

Domestic Yorkshire swine were supplied by Dalessio's Stock Farm (Plumville, PA, U.S.A.). The animals had been raised in confined housing to protect the skin from abrasion and were maintained in the Central Animal Facility of the University for 2 weeks prior to use. Here they received water and a complete swine grower diet *ad libitum*, and were housed in pens with controlled temperature (20–23°C) and lighting (12 h light: 12 h dark).

### *Explant preparation*

For each experiment, skin was excised from the paravertebral region of a young Yorkshire swine

(11–15 kg). The animal, which had received no previous anaesthesia or treatment, was anaesthetized with ketamine (300 mg, i.m.) and methoxyfluorane (3%, open mask). The skin on the back was prepared for excision as follows: hair was removed with fine barber's clippers and a razor. The skin was washed with neutral soap and water, then once with 10% povidone-iodine, twice with 70% methanol, and three times with sterile distilled water. Skin sheets, 50 × 22 mm, were excised aseptically with a Castro-Viejo electro-keratome set to cut at a depth of 0·2 mm. The sheets were placed in RPMI 1640 medium containing 2% AB/AM and kept at 4°C prior to dissection.

The explants were prepared within 24 h after surgical excision, according to the methods originally developed by Halprin, Lueder and Fusenig (1979) and adapted to culture of young adult porcine skin by Hammar and Halprin (1981). The skin sheets were kept moist with medium throughout the dissection procedure. Each sheet was examined macroscopically and areas of uniform thickness selected. Individual explants (1–2 mm squares) were prepared with a sharp scalpel, taking care to cut clean edges perpendicular to the surface. Explants were placed dermal-side down in 35 mm plastic culture dishes, four explants per dish. To distribute the tissue specimens uniformly, as each culture dish of explants was prepared, it was assigned to the next treatment group in the sequence. A minimum of 10 dishes per treatment group were prepared for each experiment. In every experiment, control cultures were included to correct for variation in different skin samples. After the explants had been air-dried for 15–30 min to obtain adherence to the plastic culture dish, 2 ml of culture medium containing the appropriate test material and 2% AB/AM was added to each dish. The explant cultures were incubated at 37°C with 5% $CO_2$.

### *Evaluation of explant outgrowth*

Cultures were examined for onset of epidermal cell outgrowth. Once outgrowth was observed (day 3 in these experiments) the longest radius on each growing side of each explant was measured using a phase contrast microscope (× 100) fitted with a micrometre eyepiece. The measurements were repeated on days 5 and 7. The change in the radius for each side was calculated and the maximum difference in the radius between days 3 and 7 was used to calculate the rate of outgrowth for each explant, and the mean (± SEM) was calculated for each treatment group. Statistical analysis was performed using analysis of variance with repeated measures (Winer, 1971), *post hoc* comparisons of individual differences using Scheffe's procedure (Winer, 1971), and Dunnett's procedure for multiple comparisons of several treatments with a control (Dunnett, 1955). An overall significance level of 0·05 was used for the multiple comparisons procedure. Explant viability was measured as the number of explants showing outgrowth on day 3. Groups were compared for differences in viability using the chi-square test (Sokal & Rolff, 1981).

### *Platelet homogenate fraction*

Human platelets (one unit of platelets was concentrated approximately × 10 from one unit, or pint, of whole blood) from the Blood Bank were centrifuged at 400 ***g*** for 10 min to sediment residual red blood cells. The supernatant platelet-rich plasma was centrifuged at 1500 ***g*** for 10 min to yield a platelet pellet and supernatant platelet-poor plasma. The plasma was discarded and the packed platelets were gently resuspended in an isotonic buffer (100 mM KCl, 50 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM EDTA, pH 7·4) (Salganicoff *et al.*, 1975). This platelet suspension was centrifuged at 1500 ***g*** for 10 min to yield washed platelets. The platelets were resuspended in a small volume of the same buffer (1 ml/unit of platelets) and homogenized with a Tekmar Tissumizer at 10 000 rpm for 30 s. The homogenate was centrifuged at 15 000 *g* for 10 min to sediment cellular debris which was discarded. The supernatant was sterilized by passage through a 0·45 μm Millex filter (Millipore Corporation, Bedford, MA, U.S.A.). This platelet homogenate fraction (PHF) was assayed for protein concentration with the Bio-Rad rapid protein assay (Bradford, 1976).

### *Heat inactivation of platelet homogenate fraction*

PHF was added to serum-free medium at a concentration of 260 μg protein/ml. The suspension was incubated in a boiling water bath with mixing for 2 or for 5 min, then cooled rapidly in an ice bath. After filtration through a 0·45 μm Millex filter, the heat-inactivated PHF concentrate was added to medium with or without FBS to produce a 10-fold dilution of PHF (approximately 26 μg protein/ml, some denatured protein being lost during filtration).

### *Fractionation of platelet homogenate fraction*

PHF was filtered through a YM-type membrane (Amicon, Danvers, MA, U.S.A.) which excludes molecules larger than approximately 30 kd. The ultrafiltration method was previously calibrated using radiolabelled protein standards mixed with PHF to confirm that molecules smaller than 30 kd were eluted through the filter and that molecules larger than 30 kd were retained.

## RESULTS

### *Effect of platelet homogenate fraction on explant outgrowth*

The explants were cultured in medium containing varying concentrations of PHF and FBS. A total of 30 culture dishes were prepared for each of the eight treatment groups, with four explants per culture dish, giving a total of 120 explants per group. Onset of outgrowth was observed on day 3. The measurements of epidermal cell outgrowth on days 3, 5 and 7 are shown in Table 1. Except for Group 1 (negative control), each group contained 26–69% viable explants (i.e. explants which showed outgrowth). Since the non-growing (non-viable) explants were

TABLE 1. The effects of platelet homogenate fraction (PHF) and foetal bovine serum (FBS) on explant outgrowth

| | Group No | PHF (μg/ml) | Viability (%)* | Outgrowth μm (mean ± SEM) | | |
|---|---|---|---|---|---|---|
| | | | | Day 3 | Day 5 | Day 7 |
| Without FBS | 1 | 0 | 2 | 366 ± 79 | 516 ± 17 | 522 ± 11 |
| | 2 | 26 | 69 | 566 ± 24 | 728 ± 24 | 804 ± 25 |
| | 3 | 130 | 45 | 522 ± 26 | 692 ± 29 | 818 ± 32 |
| | 4 | 260 | 55 | 445 ± 26 | 633 ± 18 | 751 ± 33 |
| With 5% FBS | 5 | 0 | 67 | 620 ± 21 | 761 ± 23 | 851 ± 20 |
| | 6 | 26 | 30 | 224 ± 13 | 314 ± 17 | 390 ± 20 |
| | 7 | 130 | 26 | 210 ± 14 | 265 ± 18 | 340 ± 24 |
| | 8 | 260 | 37 | 242 ± 17 | 290 ± 15 | 383 ± 24 |

* Viability $= n/T \times 100\%$, where $n$ = number of explants growing on day 3 and $T$ = total number of explants prepared (120).

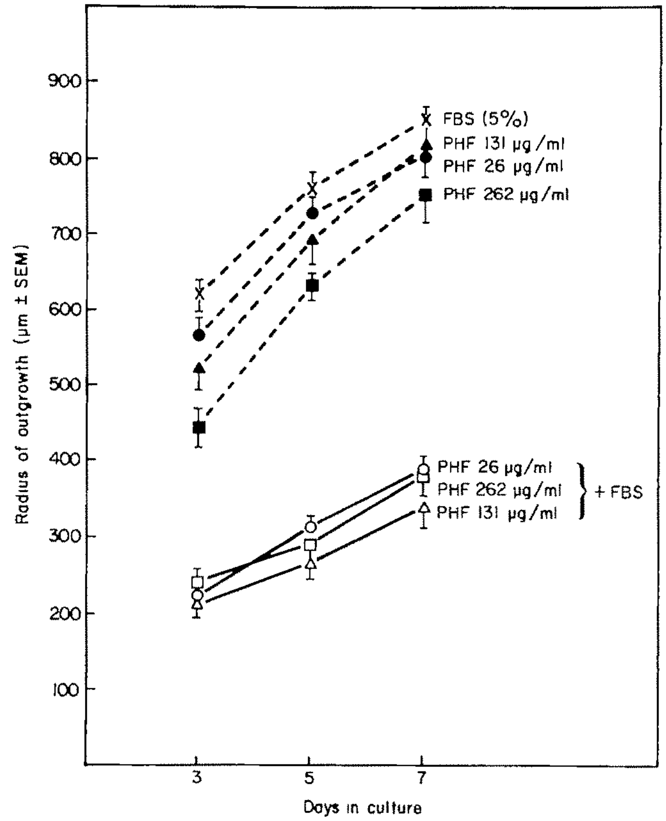

FIGURE 1. Epidermal cell outgrowth from porcine skin explants. Points are means for numbers of explants growing (± SEM) (see Table 1).

distributed evenly throughout each group rather than being clustered together in the same dish or dishes, it is unlikely that a loss of viability was due to a culture dish effect. Viability in Groups 2, 3 and 4 (PHF alone) ranged from 45% to 69%, which was not significantly different from the viability of 67% in Group 5, the positive control with 5% FBS. However, viability was significantly lower in Groups 6, 7 and 8 grown with PHF plus FBS (26–37%, $P < 0{\cdot}001$). Only 2% (two explants) showed outgrowth in medium without PHF or FBS (negative control group). Their outgrowth stopped after day 5.

The explant outgrowth measurements are shown in Figure 1. PHF evoked total outgrowth very similar to the outgrowth obtained with 5% FBS. The outgrowth with 26 µg/ml PHF was almost the same as the outgrowth with FBS at every time point. With 260 µg/ml PHF, outgrowth was significantly lower on days 3 and 5 compared with the other concentrations of PHF ($P = 0{\cdot}05$). By repeated measures analysis of variance, it was shown that there was a dose-dependent PHF effect. There were interactions between treatment and time on day 7 that were not unexpected as the rate of outgrowth also decreased during later stages of explant growth in the FBS controls. The outgrowth from explants grown with PHF plus FBS was similar for all three PHF concentrations, and was much less than with any concentration of PHF alone in both the radius of the epidermal sheet (total outgrowth) and the rate of outgrowth ($P < 0{\cdot}001$).

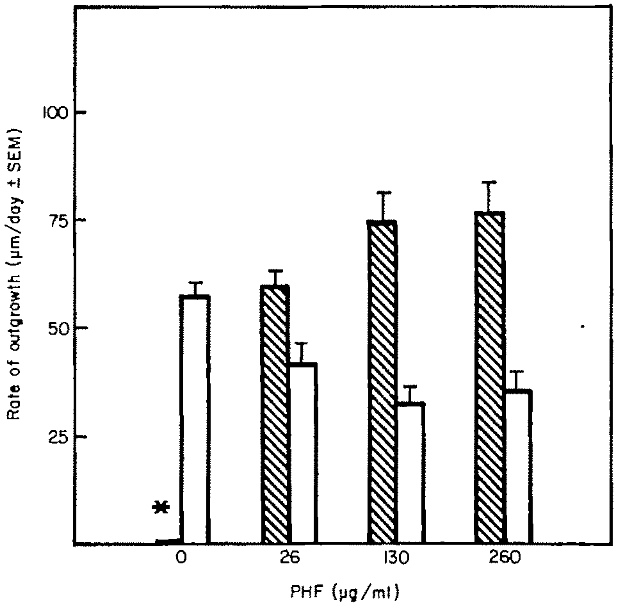

FIGURE 2. Rates of epidermal cell outgrowth from porcine skin explants between days 3 and 7 at varying concentrations of PHF with and without 5% FBS*. The negative control group was non-viable; 2/120 explants, however, exhibited limited outgrowth which was attributed to residual nutrients in the explant tissue. Means ± SEM for numbers of explants in each group growing, see Table 1. ▨ 0% FBS, ☐ 5% FBS.

In Figure 2, the rates of outgrowth between days 3 and 7 are shown for different concentrations of PHF with and without FBS. There was a concentration-dependent increase in the rate of outgrowth with PHF between 26 and 130 µg protein/ml which reached a plateau between 130 and 260 µg/ml. The rates of outgrowth were lower with PHF plus FBS than with FBS or PHF alone. This inhibition was not PHF concentration-dependent within the range tested (26–260 µg/ml).

Cultures were examined under a dark field and phase contrast microscope and were found to have structural features characteristic of epidermal sheets from explants (Hammar & Halprin, 1981; Flaxman & Harper, 1975). The cells in the epidermal sheets were typical of keratinocytes on the basis of their morphology and growth kinetics. Cultures grown with PHF (Figure 3a and b) had epidermal sheets similar to FBS controls—the sheet was multilayered near the explant, tapering to a single cell layer at the growing edge with many 'stretched' epidermal cells (Halprin *et al.*, 1979). Cultures grown with PHF plus FBS (Figure 3c and d) had abnormal features compared with FBS controls. The epidermal sheet was thick and multilayered throughout, and there was no tapering towards a monolayer as is normally found. The cells at the leading edge were not 'stretched' or spread but were rounded and piled up. There was no morphological evidence of extensive cell death, since the cells remained adherent to each other and the epidermal sheet remained intact throughout the 7 to 10-day period of observation.

### *Dose-dependence of explant viability and outgrowth*

Serial dilutions of PHF were tested to determine the lower limits of the PHF effect on explant viability and outgrowth. The results shown in Table 2 indicate that PHF supported explant

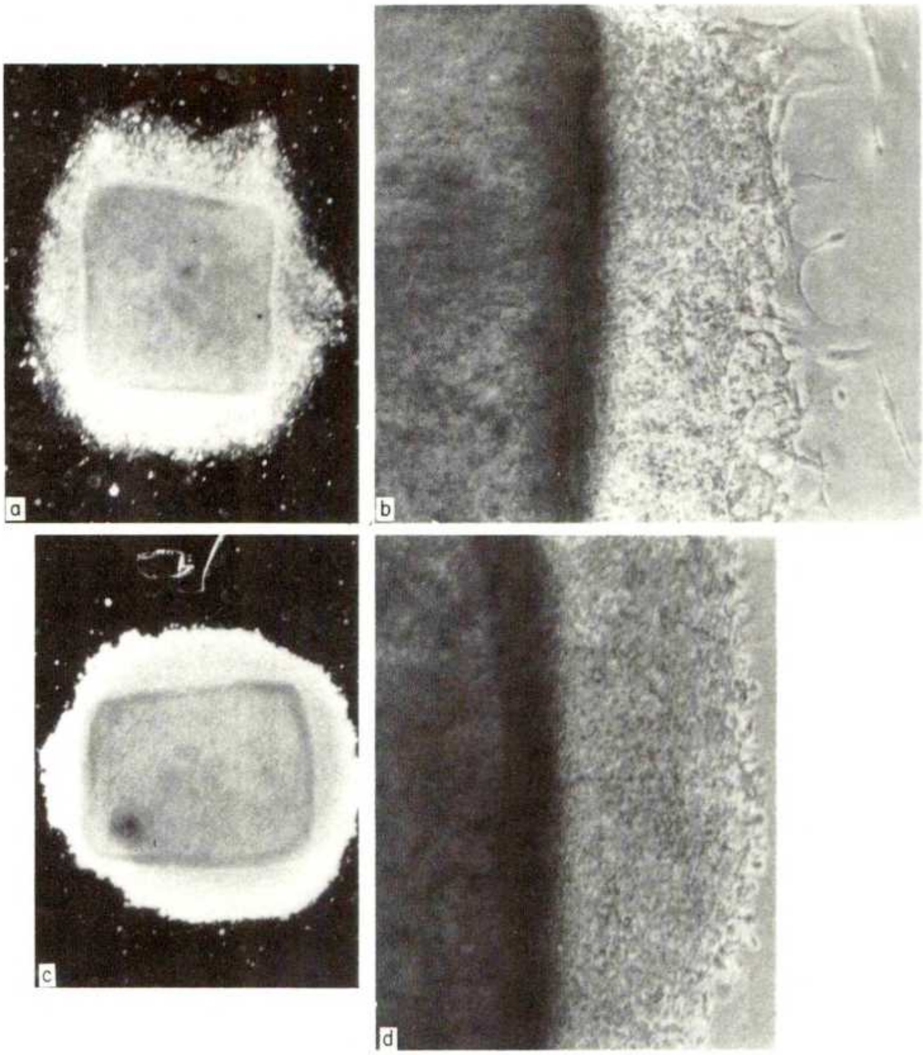

FIGURE 3. Photomicrographs of porcine skin explants on day 7. (a) explant cultured in 26 μg/ml PHF. The original explant (central square of skin) is seen surrounded by the epidermal sheet (outgrowth). Note gradation in brightness of the epidermal sheet indicating gradual thinning to a monolayer at the growing (outer) edge. (Dark field; original × 31·2). (b) Same explant as (a) viewed under phase contrast. Note thick, multilayered sheet near the explant (left) tapering to monolayer of 'stretched' cells at the growing edge (right). (Original × 125). (c) Explant cultured in 130 μg/ml PHF + 5% FBS. Note uniformly thick epidermal sheet. (Dark field; original × 31·2). (d) Same explant as (c) viewed under phase contrast. Note absence of flattened and 'stretched' cells at growing edge (right). All the cells are rounded and piled up. (Original × 125).

TABLE 2. Effect of PHF and FBS concentration on explant viability and outgrowth

| Treatment | Viability (%)* | Rate of outgrowth µm/day (± SEM) | % of control† |
|---|---|---|---|
| PHF only (µg/ml) | | | |
| 0·026 | 0 | 0 | 0 |
| 0·26 | 0 | 0 | 0 |
| 2·6 | 58 | 135 (± 10) | 41 |
| 26 | 64 | 290 (± 20) | 88 |
| 130 | 70 | 370 (± 13) | 109 |
| FBS only (%) | | | |
| 0 | 5 | 165 (± 1·6) | 50 |
| 5 | 84 | 330 (± 3·3) | 100 |

* See Table 1 ($T=40$).

† Outgrowth is shown relative to the rate obtained with 5% FBS.

TABLE 3. The effect of platelet-derived growth factor (PDGF) on explant outgrowth

| PDGF* (units/ml) | Rate of outgrowth 0% FBS | 5% FBS µm/day | (± SEM) | % of control† |
|---|---|---|---|---|
| 0 | 0 | 216 | (±9) | 100 |
| 0·2 | 0 | 249 | (±15)‡ | 115 |
| 1·0 | 0 | 260 | (±9)‡ | 120 |
| 5·0 | 0 | 268 | (±4)‡ | 124 |

* 1 unit PDGF = the amount which evokes a response in fibroblasts equal to that of 5% FBS.

† Outgrowth is shown relative to the rate obtained with 5% FBS.

‡ Rate of outgrowth was significantly greater than with FBS alone, as determined by Dunnett's procedure, using an overall significance level of 0·05.

viability at a concentration of 2·6 µg/ml and above. The rate of outgrowth achieved with PHF reached the same rate as the FBS control at a PHF concentration between 26 and 130 µg/ml.

### *Effect of platelet-derived growth factor on explant outgrowth*

Explants were cultured in serum-free medium containing commercial partially-purified PDGF, at concentrations of 0·2, 1·0 and 5·0 units/ml, one unit being the amount evoking a response in fibroblasts equal to that evoked by 5% FBS. The results are summarized in Table 3. PDGF did not support epidermal cell outgrowth in the absence of FBS. In the presence of 5% FBS, PDGF produced a slight but significant stimulation of epidermal cell outgrowth at 0·2, 1·0 and 5·0 units/ml.

### *Effect of heat on platelet homogenate fraction*

Explants were cultured in medium containing heat-treated PHF, and outgrowth was compared with that in PHF-treated and FBS-treated groups. The results, shown in Table 4, indicate that the stimulatory activity of PHF was stable to heat at 100°C for 2 min, but was destroyed by heating at 100°C for 5 min. During the course of these studies, it was found that the inhibitory effect of PHF plus FBS was not obtained with every PHF preparation. The results of the experiment presented in Table 4 show that PHF plus FBS did not inhibit viability or outgrowth, whereas inhibition was found in two previous experiments.

### *Fractionation of platelet homogenate fraction by molecular size*

PHF was separated into two fractions by filtration through a membrane that excluded molecules larger than 30 kd. The fractions were tested for their effects on explant outgrowth at a concentration equivalent to 26 μg/ml original PHF and the results are shown in Table 5. The 'high molecular weight' fraction (> 30 kd) supported explant viability and outgrowth comparable to the original PHF. The 'low molecular weight' fraction (< 30 kd) did not support viability as well as original PHF, and the explants that were viable showed a slower rate of

TABLE 4. The effect of heat inactivation on PHF-supported explant outgrowth

| | | Rate of outgrowth | |
|---|---|---|---|
| Experimental conditions | Viability (%)* | μm/day(± SEM) | % Control† |
| No addition | | | |
| (Negative control) | 25 | 95(±6)‡ | 44 |
| 5% FBS | | | |
| (Positive control) | 98 | 216(±9) | 100 |
| PHF (26 μg/ml) | 78 | 190(±13) | 88 |
| (130 μg/ml) | 70 | 236(±9) | 109 |
| PHF heat-treated | | | |
| 100°C for 2 min (26 μg/ml) | 58 | 175(±15)‡ | 81 |
| 100°C for 5 min (130 μg/ml) | 15 | 141(±11)‡ | 65 |
| 5% FBS | | | |
| + PHF (26 μg/ml) | 98 | 240(±17) | 111 |
| + heat-treated PHF (26 μg/ml) | | | |
| 100°C for 2 min | 85 | 264(±15)‡ | 122 |
| 100°C for 5 min | 70 | 232(±13) | 107 |

* See Table 1 ($T = 40$).

† Outgrowth relative to the rate obtained with 5% FBS.

‡ Significantly different from FBS positive control, as determined by Dunnett's procedure, using an overall significance level of 0·05.

TABLE 5. Effect of low and high molecular weight fractions of PHF on explant viability and outgrowth

| Fraction | Percentage total protein | Viability (%)* | Rate of outgrowth |  |
|---|---|---|---|---|
|  |  |  | μm/day (± SEM) | % of control† |
| PHF (not fractionated) | 100 | 64 | 240(± 13) | 100 |
| Low molecular weight (<30 kd) | 10‡ | 20 | 139(± 13) | 58 |
| High molecular weight (>30 kd) | 85‡ | 62 | 220(± 11) | 92 |

* See Table 1 ($T=40$).

† Outgrowth relative to the rate obtained with unfractionated PHF.

‡ Total recovery in the two fractions = 95%.

outgrowth. These results indicate that the activity of PHF in promoting epidermal cell outgrowth is due primarily to macromolecular components approximately 30 kd or larger.

## DISCUSSION

This study provides evidence that human platelets contain factors that support epidermal cell viability, initiate epidermal outgrowth and stimulate the rate of outgrowth from porcine skin explant cultures without serum supplement.

An explant was considered viable if it showed any epidermal cell outgrowth by day 3. The results demonstrate that a factor (or factors) in PHF, added to the culture medium, supported explant viability similar to that in positive control cultures grown in culture medium with 5% FBS. The limited outgrowth (2–5%) in unsupplemented medium (negative control) may have been due to residual nutrients within the explant tissue. The factor in PHF responsible for explant viability is unlikely to be PDGF since commercial PDGF added alone to the medium did not produce viable explant outgrowth. These experiments show that epidermal cell migration could be initiated in PHF-supplemented medium without serum. Since the initiation of explant outgrowth (epidermal cell migration) requires attachment and spreading, PHF must have contained factors that permitted or facilitated both of these events. PHF (26 μg/ml) initiated outgrowth as effectively as 5% FBS. However, the radius of outgrowth on day 3 was inversely proportional to the PHF concentration (see Fig. 1). It may be that the concentration of outgrowth initiation factor in PHF exceeded the effective range and actually inhibited initiation. This lower initial rate of outgrowth at higher concentrations may have been due to the absence in PHF of a factor for outgrowth initiation that was present in FBS, thus making explants cultured in PHF slower to start growing.

PHF stimulated outgrowth in a concentration-dependent manner. This stimulatory effect was probably not related to PDGF since the explants did not grow in PDGF-supplemented medium. The demonstration by Heldin *et al.* (1981) that an epithelial cell line lacked cell surface PDGF receptors supports the conclusion that PDGF does not affect epithelial cells directly. It is possible that PDGF may act synergistically with another factor present in PHF (and serum) to stimulate epidermal cell outgrowth. This idea is supported by our finding that PDGF plus FBS stimulated epidermal outgrowth. The possibility that PDGF plus FBS has an effect on epithelial cells is also suggested by the report of Aso *et al.* (1980) that thrombin-treated platelets plus plasma increased the size and number of colonies in keratinocyte cultures. However, both our study and that of Aso *et al.* (1980) used PDGF that may have contained at least trace amounts of other factors.

In addition to PDGF, other growth factors which have been found in platelets include platelet basic protein (Paul *et al.*, 1980), connective tissue activating peptide III (Castor *et al.*, 1977), transforming growth factor-beta (Childs *et al.*, 1982), an epidermal growth factor variant (Assoian *et al.*, 1984; Oka & Orth, 1983) and other factors (Westermark *et al.*, 1983). These factors have been found to affect connective tissue cells but not epithelial cells. Therefore, ours is the first demonstration of an effect of platelet-derived factors on epidermal cell outgrowth. One or more of the factors in PHF active for connective tissue cells may have influenced the epidermal cells indirectly through cells in the dermis. This is a possibility that cannot be ruled out in explant cultures since they contain a portion of the papillary dermis.

Factors that influence epidermal cells directly have been found in plasma and serum; epidermal growth factor (Savage & Cohen, 1972), epibolin (Stenn, 1981), serum spreading factor (Barnes *et al.*, 1980) and fibronectin (Yamada & Olden, 1978). One or more of these may also be associated with platelets. Since the stimulatory effect of PHF was stable to treatment at 100°C for 2 min, it probably is not fibronectin, which is heat labile if heated to 80°C for 2 min. The finding that the activity was destroyed by heating at 100°C for 5 min eliminates epidermal growth factor, connective tissue activating peptide III and transforming growth factor-beta, but leaves the possibility that epibolin or serum spreading factor is responsible for the stimulatory effect. The activity of PHF is not distinguishable from epibolin or serum spreading factor on the basis of heat stability or molecular weight. Epibolin was first identified as the factor that causes epiboly, the spreading and migration of epidermal cells around the dermis in floating explants. Epibolin has been found in plasma and serum in approximately equivalent amounts. It has not been reported in platelets but one explanation for our observations is that epibolin is associated with platelets. Serum spreading factor has been purified from serum and has a high degree of homology with and may be related to epibolin. It is also possible that we are observing a 'new' platelet-derived factor which influences epidermal cells.

PHF and FBS together resulted in inhibition of viability, decreased total outgrowth and slower rates of outgrowth. FBS contains factors released by platelets, and when combined with PHF, may have inhibited explant outgrowth by producing either an increased amount of an inhibitor, excess amounts of factors stimulatory for attachment, spreading or migration that are inhibitory at higher concentrations, inhibitory products from the combination of PHF and FBS, or an increased amount of a cytotoxin that kills a portion of the epidermal cell population. The fourth possibility can probably be eliminated because miroscopic examination showed no cell death, but rather a piling up of cells in the epidermal sheet. The piled up cells did not exhibit a migratory morphology but rather were rounded and poorly attached to the dish. The possibility of a factor being stimulatory at low concentration and inhibitory at higher concentrations is plausible since this has been observed with higher concentrations of serum spreading factor (Barnes *et al.*, 1980) and transforming growth factor-beta (Roberts *et al.*, 1985). The inhibitory effect was not observed in every preparation of PHF, suggesting that it either was not universally present in platelets or was very labile and was lost during processing.

The importance of platelets in the wound healing process is a subject of active investigation (Seppa *et al.*, 1982; Ross, 1980; Deuel *et al.*, 1982; Knighton *et al.*, 1982). It is known that platelet-derived factors stimulate events crucial to normal dermal wound healing (fibroplasia, collagen biosynthesis and angiogenesis). However, an effect on epidermal wound healing has not been proved, although the possibility has been suggested (Aso *et al.*, 1980). In the present study, a platelet fraction was found to support and stimulate epidermal cell outgrowth from skin explants. These results show that platelet factors can influence epidermal cell attachment and migration, either directly or through dermal interactions. These results support the hypothesis that platelets play an important role in the healing process, including epidermal as well as dermal healing.

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# *In vivo* induction of Ia antigens on rat keratinocytes by $\gamma$-interferon

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## SUMMARY

Recombinant rat IFN-$\gamma$ was found to induce Ia antigen expression on rat keratinocytes *in vivo*. Strong expression of Ia antigen on keratinocytes of rat ears was produced after intradermal injections of 10 000 U IFN-$\gamma$ once daily on 3 successive days. The expression was still pronounced 2 days after the last injection, but had disappeared 4 days later.

The expression of class II transplantation antigens (HLA-D in man and Ia in mouse) is limited, in normal epidermis, to the immunocompetent Langerhans cells (Klareskog *et al.*, 1977; Rowden, Lewis & Sullivan, 1977). The class II antigens function primarily as restriction elements recognized by T cells of the helper/inducer subset (Schwartz, 1984). Inducible expression of class II antigens on keratinocytes has been reported in different skin disorders and in experimental situations associated with infiltrates of T lymphocytes and in which cellular immune reactions are presumed to take place (Tjernlund, 1978, 1980; Lampert, Suitters & Chrisholf, 1981; Mason, Dallman & Barclay, 1981; Barclay & Mason, 1982; Suitters & Lampert, 1982; Scheynius & Tjernlund, 1984; Volc-Platzer *et al.*, 1984; Smolle, 1985). The infiltrating T cells may release $\gamma$-interferon (IFN-$\gamma$), which is a potent inducer of class II antigens in a variety of cell types, including keratinocytes (Steeg *et al.*, 1982; Pober *et al.*, 1983; Basham *et al.*, 1984a). The capacity of IFN-$\gamma$ to induce the synthesis and expression of class II antigens on keratinocytes has been shown in *in vitro* cultures (Basham *et al.*, 1984a; Volc-Platzer *et al.*, 1985), but not *in vivo*. We therefore set up an experimental system to determine whether IFN-$\gamma$ injected into rats could mediate the expression of Ia antigens on keratinocytes.

## METHODS

DA rats originally obtained from Anticimex (Stockholm, Sweden) were bred in our own breeding facilities and used at 2–6 months of age. Recombinant rat IFN-$\gamma$ (200 000 U/ml) was

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produced and purified as described previously (Dijkema *et al.*, 1985) and stored at − 70°C. The antiviral activity of rat IFN-$\gamma$ was determined by the cytopathic effect reduction assay (Armstrong, 1971): the assay was standardized against a laboratory standard of rat IFN-$\gamma$. 1000–10 000 U IFN-$\gamma$ in 0·1 ml phosphate-buffered saline (PBS) pH 7·4, was injected intradermally into the dorsal surface of the left ear once or on two or three successive days. The right ear was injected with 0·1 ml PBS as a control. Groups of at least two rats were killed at various times after the injections. The ears were cut off at the base, snap frozen in isopentane and stored at − 70°C.

*Identification of Ia antigen expressing cells*

Acetone-fixed cryostat sections, 4 μm thick, were processed for peroxidase-anti-peroxidase (PAP) staining according to the method of Sternberger (1979). The sections were cut through the centre of each ear extending from the top to the base. Mouse monoclonal antibodies, clones

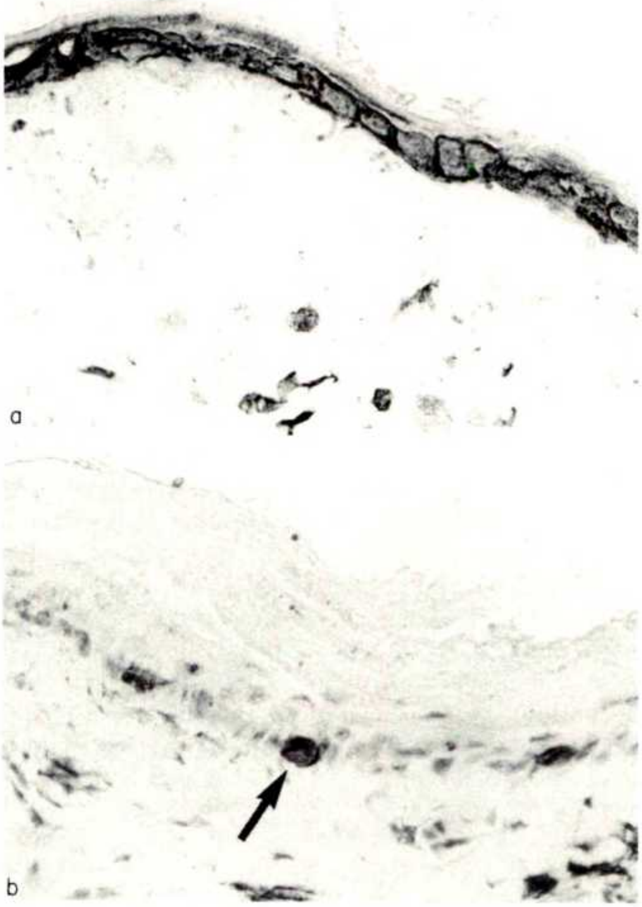

FIGURE 1. Immunoperoxidase staining of OX 6-positive cells on frozen sections of rat ear skin. (a) After intradermal injection of 10 000 U IFN-$\gamma$ in 0·1 ml PBS once daily for 3 consecutive days and biopsied 3 h after the last injection. Note the expression of Ia antigens by keratinocytes. (b) An ear injected similarly with 0·1 ml PBS. Arrow indicates an epidermal cell expressing Ia antigen.

OX 6 and OX 17 (Ig content $\geqslant$2 mg/ml) were obtained from Sera-Lab (Cambridge, U.K.). OX 6 antibodies ($IgG_1$, diluted 1:1920) react with a common determinant of rat Ia antigens corresponding to I-A coded molecules in mice (McMaster & Williams, 1979), whereas OX 17 antibodies ($IgG_1$, diluted 1:1000) recognize a monomorphic determinant on the $\alpha$ chain of rat Ia antigen, homologous with the mouse Ia-E product (Fukumoto, McMaster & Williams, 1982). Goat anti-mouse IgG was absorbed on Sepharose-coupled rat IgG and used as a secondary antibody (diluted 1:20). Preformed complexes of horse-radish peroxidase and mouse-monoclonal anti-horse-radish peroxidase antibody (diluted 1:64) were obtained from Dakopatts (Copenhagen, Denmark). The peroxidase reaction was developed with carbazole (Kaplow, 1975) and the sections counterstained with haematoxylin. The dilutions of the antibodies were determined using sections from normal lymph nodes and skin. Controls without the primary antibodies or with irrelevant antibodies gave no staining.

Each ear was also processed for haematoxylin and eosin staining for the estimation of cell infiltrates.

## RESULTS

As can be seen in Figure 1a, intradermal injections of IFN-$\gamma$ induced Ia antigen expression on keratinocytes. In the epidermis of the ears injected with PBS, Ia antigen expression was limited in all cases to a few dendritic cells, presumed to be Langerhans cells (Fig. 1b).

The optimal induction of Ia antigens on keratinocytes was obtained with 10 000 U IFN-$\gamma$ given once daily for 3 consecutive days, although two injections of 1000 U IFN-$\gamma$ was enough to induce Ia antigens on basal keratinocytes (Fig. 2). Three hours after the third injection of 10 000 U IFN-$\gamma$ there was clear expression of Ia antigens on all keratinocytes in an area corresponding to about one-half to two-thirds of the dorsal surface of the ear. The expression of Ia antigens on keratinocytes was still pronounced 2 days after the last injection, but had disappeared 4 days later (Fig. 2). Staining of the keratinocytes predominantly involved the cell membranes, but there was also faint staining within the cytoplasm of the cells (Fig. 1a).

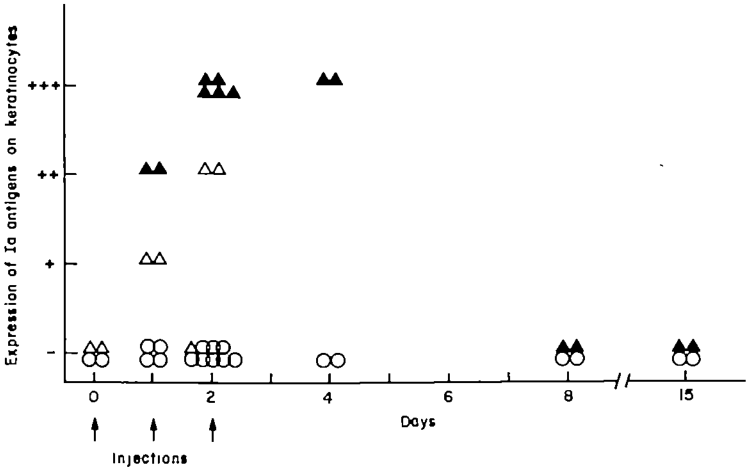

FIGURE 2. Time course of Ia antigen expression on keratinocytes after intradermal injections of IFN-$\gamma$ 1000 U (△) or 10 000 U (▲), or PBS (○) once or on two or three consecutive days. Expression of Ia antigens on keratinocytes was measured by immunoperoxidase staining of OX6-positive cells on frozen sections of the ears and graded as: –, absent; +, patches in the basal layer; + +, detectable in the whole basal layer; and + + +, involving the full thickness of the epidermis. Each point represents the average value from three or four different sections of an individual ear. On days 0, 1 and 2 the animals were killed 3 h after the last injection.

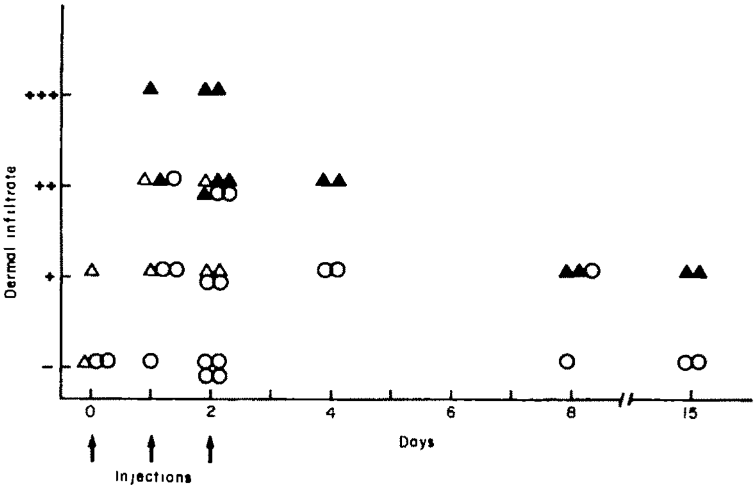

FIGURE 3. Density of cells infiltrating the dermis after intradermal injections of IFN-γ 1000 U (△), or 10000 U (▲), or PBS (○) once or on two or three consecutive days. Degree of dermal infiltration in haematoxylin and eosin stained sections graded as: –, none; +, small; + +, moderate; and + + +, large. Each point represents the average value from two to four different sections of an individual ear. On days 0, 1 and 2 the animals were killed 3 h after the last injection.

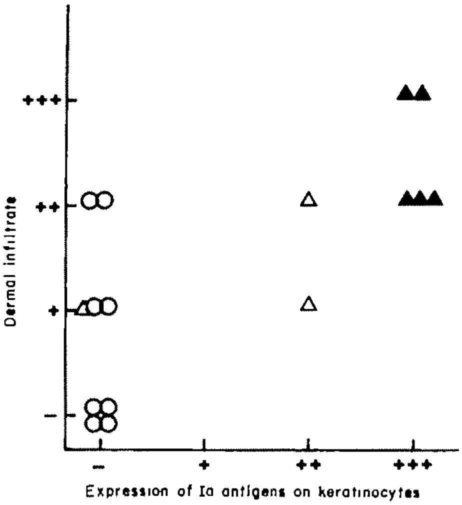

FIGURE 4. Relationship between the expression of Ia antigens on keratinocytes and the degree of dermal cell infiltration after three intradermal injections of IFN-γ, 1000 U (△), or 10 000 U (▲), or PBS (○). Grading of expression of Ia antigens on keratinocytes and degree of dermal cell infiltration are the same as in Figures 2 and 3. Each point represents the average value of two to four different sections of an individual ear.

The injections usually resulted in a dermal infiltrate, as judged by haematoxylin and eosin staining. The largest degree of infiltration was observed after the higher dose of IFN-γ (Fig. 3). Although the injections with PBS could give rise to a small to moderate degree of infiltration, these were never followed by the expression of Ia antigens on keratinocytes (Fig. 4). Many of the cells infiltrating the dermis were OX 6-positive in both the PBS and IFN-γ injected ears.

The presence of OX 17 reactivity corresponding to mouse Ia-E antigens on keratinocytes was less pronounced than OX 6 (Ia-A) staining and mainly observed on those ears that exhibited the strongest expression of Ia-A antigens.

## DISCUSSION

The present study shows that intradermal injections of IFN-γ can induce Ia antigen expression on keratinocytes *in vivo*. Our observation is thus in agreement with previous results obtained with keratinocytes cultured in the presence of IFN-γ (Basham *et al.*, 1984a; Volc-Platzer *et al.*, 1985). These earlier *in vitro* studies have shown that the keratinocytes synthesize their own class II antigens (Volc-Platzer *et al.*, 1985), and furthermore that the induction of class II antigens on keratinocytes is independent of Langerhans cells (Basham *et al.*, 1984a). Langerhans cells themselves are unaffected by IFN-γ (Basham *et al.*, 1984b).

Whether the larger number of cells infiltrating the dermis in the ears injected with the higher dose of IFN-γ was due to a chemotactic response to exogenous IFN-γ or was the result of a secondary response to the large degree of induction of class II antigens on the keratinocytes, cannot be decided from the present data. Nor can the possibility be excluded that endogenous IFN-γ might have contributed to the expression of class II antigens on keratinocytes. In the control ears injected with PBS, however, Ia antigen expression on keratinocytes was never observed, although a small to moderate degree of dermal infiltration was sometimes present.

Mason *et al.* (1981) observed that rat keratinocytes in graft-versus-host disease reacted similarly with OX 17 and OX 6 antibodies. In a later work, Barclay and Mason (1982) stated that the Ia antigen staining on keratinocytes in the contact allergic reaction, measured by Ia-A antibodies, was patchy and generally weaker than that seen in graft-versus-host disease, where the staining was heavy and continuous throughout the epidermis. It would have been interesting if they had also compared the Ia-A reactivity with that of Ia-E in the contact allergic reaction, since in our study it appeared that only those ears with a strong homogeneous reactivity with OX 6 antibodies in the epidermis reacted to a detectable degree with OX 17 antibodies.

Human keratinocytes also seem to have a differential expression of class II antigens. However, in the human system, keratinocytes are preferentially induced to express HLA-DR antigens, the human analogue of murine Ia-E antigens, and not HLA-DQ antigens corresponding to murine Ia-A antigens (Volc-Platzer *et al.*, 1984; Scheynius & Tjernlund, 1985; Volc-Platzer *et al.*, 1985; Tjernlund *et al.*, 1986). The differential expression of class II molecules is of interest since human class II antigens, like the mouse Ia-A and Ia-E antigens, differ in their contributions to the immune response. Thus, in the mouse, immune responsiveness to several antigens is associated with the expression of a particular allele of either I-A or I-E (Klein *et al.*, 1981), and in man functional differences have been reported between HLA-DR expressing monocytes, correlated with the presence or the absence of simultaneous expression of HLA-DQ antigens (Gonwa *et al.*, 1983).

Whether keratinocytes induced to express class II antigens actually participate in immune regulation has still to be elucidated. Recently, it was reported that human thyroid follicular epithelial cells with induced expression of class II antigens can present viral peptide antigens to cloned human T cells, but not the intact virus which requires processing (Londei *et al.*, 1984). A similar enhancement of antigen-presenting capacity may thus be possible in skin with class II antigen-expressing keratinocytes. On the other hand, a suppressive mechanism may also be at work since an excess of Ia molecules can inhibit T cell proliferation (Matis *et al.*, 1983). Thus, the *in vivo* model for induction of Ia antigens on keratinocytes presented here may provide a useful tool for studying the role of inducible class II antigens on keratinocytes in immune regulation.

## ACKNOWLEDGMENT

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# The *in vitro* antimicrobial effect of azelaic acid

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## SUMMARY

Various strains of cutaneous micro-organisms were tested *in vitro* for their survival rates in 0·5 mol/l (8·4% w/v) azelaic acid solution. All bacterial strains exhibited large reductions in viability (at least 40-fold) over a 24 h test period, but little response was noted with *Pityrosporum ovale*. The bactericidal effect of azelaic acid was reduced considerably in the presence of nutrients. Minimum inhibitory concentrations (MICs) and minimum bactericidal (or fungicidal) concentrations (MBCs) were also determined. MICs varied from 0·03 mol/l to 0·25 mol/l; MBCs were all either 0·25 mol/l or greater.

Azelaic acid ($HO_2\,C(CH_2)_7\,CO_2H$)has been reported to be of therapeutic value in cases of acne vulgaris when applied topically as a 15% cream (Nazzaro-Porro *et al.*, 1983). Recent trials of 20% azelaic acid cream performed in Leeds have demonstrated reductions in the population densities of both propionibacteria (32-fold) and staphylococci (224-fold) during 6 months of therapy (Bladon *et al.*, 1986). This study was undertaken to determine the *in vitro* sensitivity of the major species of cutaneous micro-organisms to azelaic acid, with particular emphasis on *Propionibacterium acnes* and *Staphylococcus epidermidis*, the organisms most often imputed in the pathogenesis of acne.

## METHODS

### *Micro-organisms*

Four strains of *Staphylococcus epidermidis* and *Propionibacterium acnes* and one strain of each of *S. capitis*, *S. hominis*, *P. granulosum* and *P. avidum* were selected from laboratory stocks. The *Pityrosporum ovale* strain was a fresh isolate. Test inocula were 2-day cultures of the staphylococci in Nutrient Broth No. 2 (Oxoid), 5-day anaerobic cultures of propionibacteria in Reinforced Clostridial Medium (Oxoid) and 3-day cultures of *Pityrosporum* in Pityrosporum broth (Table 1). All cultures were incubated at 37°C; staphylococci and *Pityrosporum* aerobically, and propionibacteria anaerobically. Aerobic liquid cultures were incubated on an orbital shaking platform set at 160 r.p.m.

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### *Azelaic acid*

Azelaic acid was provided by Schering A.G., Berlin, Germany. Stock solutions, 0·5 mol/l, were prepared by dissolving the azelaic acid in 0·1 mol/l phosphate buffer and adjusting to pH 6 with 10 mol/l NaOH. This solution was used in both cell survival and MIC experiments.

### *Microbial survival experiments*

0·1 ml of each test inoculum was suspended in 9·9 ml of 0·5 mol/l azelaic acid stock solution. A control solution containing 1 mol/l NaCl in place of azelaic acid was inoculated simultaneously. Suspensions were maintained at 30°C. Samples (0·1 ml) were taken from each suspension immediately and at suitable intervals thereafter. Serial 10-fold dilutions were made in Nutrient Broth No. 2 (Oxoid) and viable counts performed.

To display the data graphically a logarithmic transformation was applied and the proportion of organisms surviving calculated as log $N_t/N_o$, where $N_t$ = count at time $t$ and $N_o$ = initial count.

TABLE 1. The sensitivity of various cutaneous micro-organisms to azelaic acid

| Organism (and strain) | Medium | Incubation time | MCRG (mol/l) | MIC (mol/l) | MBC (mol/l) |
|---|---|---|---|---|---|
| *Staphylococcus epidermidis* (NCTC 11047) | Nutrient broth No. 2 | 48 h | 0·031 | 0·125 | >0·25 |
| *Staphylococcus epidermidis* (NCTC 11047) | Reinforced clostridial medium | 48 h | 0·031 | 0·125 | >0·25 |
| *S. capitis* (NCTC 11045) | Nutrient broth No. 2 | 48 h | 0·063 | 0·125 | >0·25 |
| *S. hominis* (CRF 572) | Nutrient broth No. 2 | 48 h | 0·063 | 0·125 | >0·25 |
| *Propionibacterium acnes* (P 37) | Reinforced clostridial medium | 7 days | 0·125 | >0·25 | >0·25 |
| *P. granulosum* (CN 5888) | Reinforced clostridial medium | 7 days | 0·063 | >0·25 | >0·25 |
| *P. avidum* (CN 6966) | Reinforced clostridial medium | 7 days | <0·001 | 0·031 | 0·25 |
| *Pityrosporum ovale* | Pityrosporum broth* | 48 h | 0·008 | 0·25 | >0·25 |

* Nutrient Broth No. 2 (Oxoid, 12·5 g); glucose (1 g); dessicated ox-bile (Oxoid, 2 g); yeast extract (Oxoid, 1·5 g); Tween 80 (Sigma, 1 ml); distilled water (1 l). pH adjusted to 6·0 before autoclaving.

MCRG, minimum concentration reducing growth (compared with a control containing no azelaic acid).

MIC, minimum inhibitory concentration.

MBC, minimum bactericidal concentration.

Values obtained in azelaic acid solution were adjusted to take into account the loss of viability occurring in corresponding control solutions according to the formula:

$$Y = (\log N_t/N_o)_{\text{azelaic acid}} - (\log N_t/N_o)_{\text{control}},$$

where $Y$ is the corrected proportion of organisms surviving. Thus, the survival curves produced represent only the effect attributable to the action of azelaic acid.

### *Minimum inhibitory concentration and minimum bactericidal or fungicidal concentration*

One strain of each species was tested. Serial 2-fold dilutions of 0·5 mol/l azelaic acid solution were made in 2 ml of the appropriate nutrient broth (pH6; formulations listed in Table 1). Each tube was then seeded with 0·02 ml of the same inoculum as used for survival experiments. After incubation for an appropriate period (see Table 1), growth was assessed by eye, and 0·1 ml of each broth showing no visible growth was spread over the surface of a suitable recovery medium

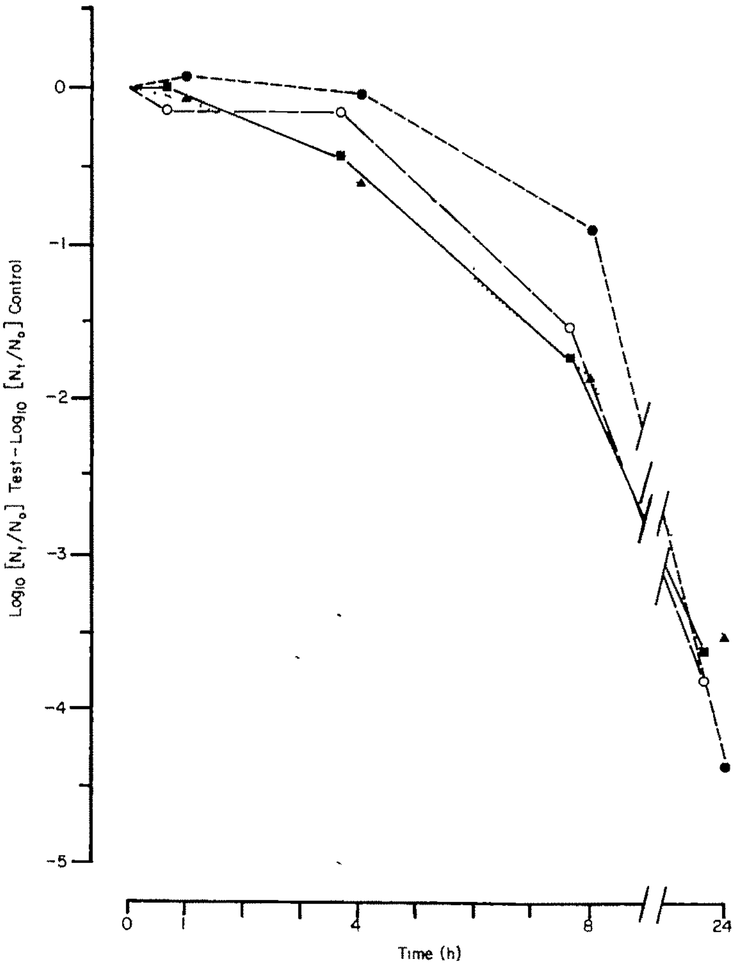

FIGURE 1. The *in vitro* effect of 0·5 mol/l azelaic acid on the viability of four strains of *Propionibacterium acnes*. Strains used: ●– – –● P37; ▲·······▲ PADH; ○——○ PAJM; △——△ CN 5936.

in order to ascertain whether viable cells remained. The minimum inhibitory concentration (MIC) was the greatest dilution showing no growth; the minimum bactericidal (or fungicidal) concentration (MBC) was the greatest dilution at which no viable organisms were recovered. The minimum concentration giving reduced growth (MCRG) was also noted.

### *Effect of nutrients*

To assess the effect of nutrients on the survival of micro-organisms in azelaic acid solution, the survival experiments with *S. epidermidis* and *P. acnes* were repeated using both the standard phosphate buffered solution of azelaic acid (0·5 mol/l) and a 0·5 mol/l solution of azelaic acid in Nutrient Broth No. 2.

The effect of different nutrient media on the MIC was assessed by repeating the MIC determination for *S. epidermidis* in Reinforced Clostridial Medium in place of Nutrient Broth No. 2.

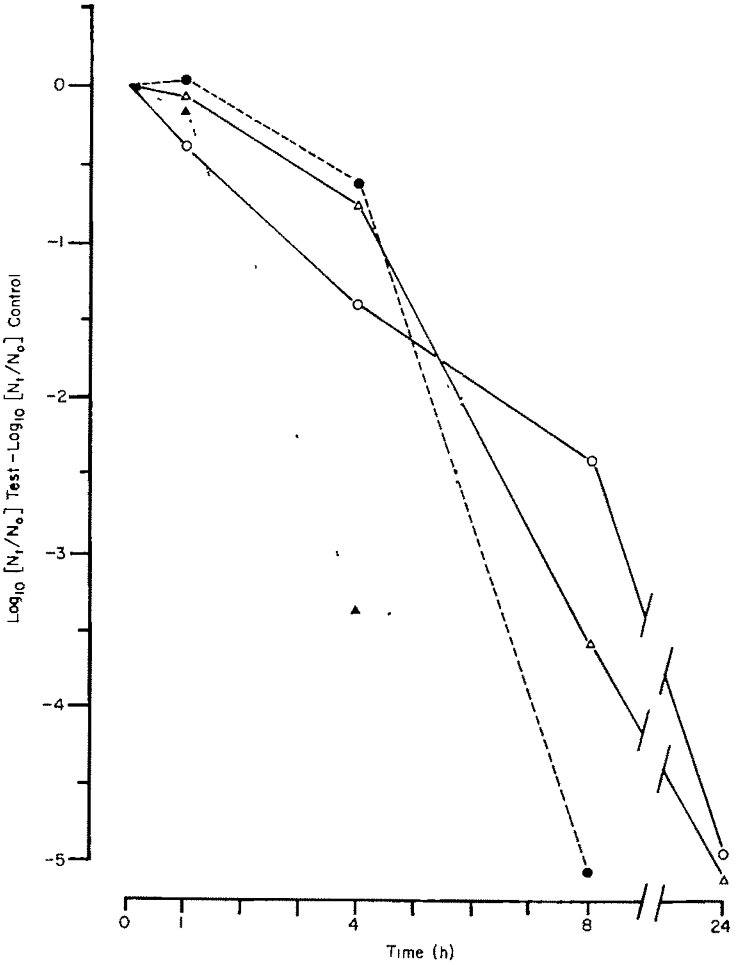

FIGURE 2. The *in vitro* effect of 0·5 mol/l azelaic acid on the viability of four strains of *Staphylococcus epidermidis*. Strains used: ●– – –● NCTC 11047; ▲· · · ·▲ SEJM; ○——○ MF20; △——△ SEAC.

## RESULTS

### *Survival curves*

The survival curves of the 13 strains tested are shown in Figures 1, 2 and 3. Different strains of the same species exhibited similar sensitivities to azelaic acid (Figs 1 and 2), but there were wide intra-generic differences which greatly outweighed inter-generic variation (Fig. 3). Repeated tests on a single test organism yielded similar death rates, but some organisms did not reproducibly exhibit a lag before loss of viability became apparent. These differences were probably due to slight variations in the physiological status of the test inocula.

Among the species most frequently isolated from acne lesions, both *S. epidermidis* and *P. acnes* were moderately sensitive (*S. epidermidis* more so than *P. acnes*), but *Pityrosporum ovale* exhibited only slight sensitivity.

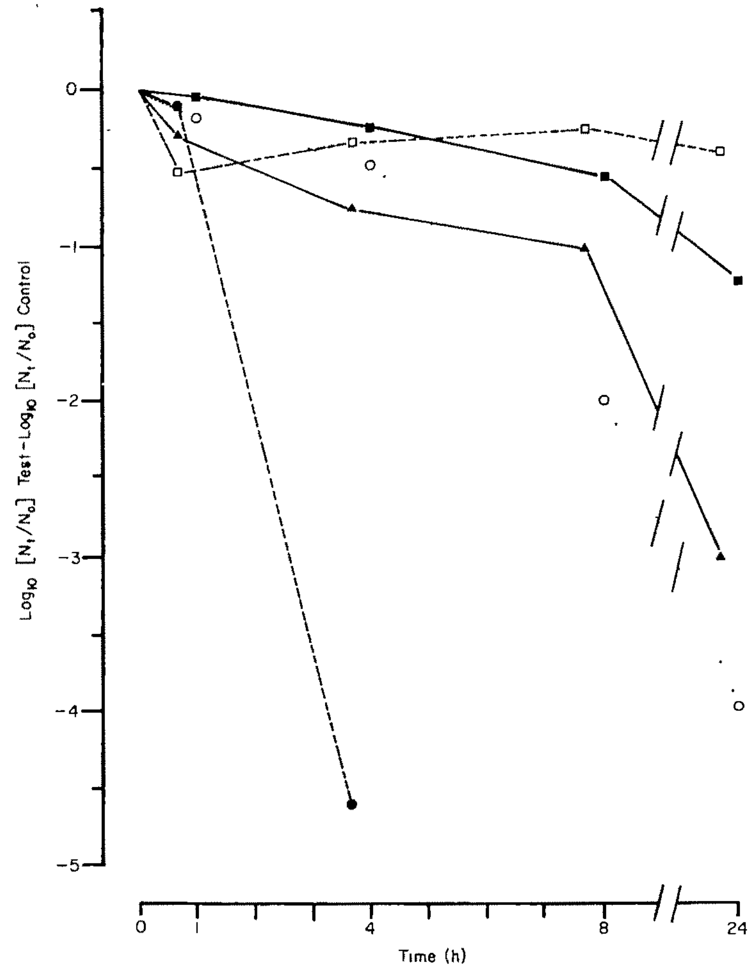

FIGURE 3. The *in vitro* effect of 0·5 mol/l azelaic acid on the viability of various cutaneous micro-organisms. ●– – –● *Propionibacterium avidum*; ○ ·· ○ *Staphylococcus capitis*; ■——■ *Propionibacterium granulosum*; □– – –□ *Pityrosporum ovale*; ▲——▲ *Staphylococcus hominis*.

### *MIC and MBC determinations*

The MIC and MBC results are listed in Table 1. The minimum concentration giving a noticeable reduction in growth (turbidity) is also listed because in some cases, particularly *P. avidum* and *Pityrosporum ovale*, this was considerably lower than the MIC. These determinations and the survival curve experiments gave similar sensitivity rankings of the organisms tested (with the exception of the MCRG for *Pityrosporum ovale*).

### *Effect of nutrients*

When azelaic acid was dissolved in nutrient broth instead of buffer, the bactericidal effect was considerably reduced; the survival rates of the four *S. epidermidis* strains were at least 50-fold higher in broth compared with buffer after 8 h exposure. Similarly, the counts of the four *P. acnes* strains were at least 10-fold higher when tested in nutrient broth. In some cases, 0·5 mol/l azelaic acid exerted only a bactericidal effect over an 8 h test period in the nutrient-rich medium (three of four *P. acnes* strains and one of four *S. epidermidis* strains).

Dissolving azelaic acid in two different nutrient media had no observable effect upon its MIC, MBC or MCBG against the *S. epidermidis* strain tested (see Table 1).

## DISCUSSION

Previous reports have suggested that azelaic acid exerts only a bacteriostatic effect upon micro-organisms (Nazzaro-Porro *et al.*, 1983). However, these investigations were performed in nutrient media. The present study confirms that azelaic acid is antimicrobial *in vitro* and demonstrates that it can exert bactericidal activity when present at high concentrations in nutrient-depleted conditions. The factors responsible for the reduction of bactericidal activity in nutrient-rich conditions have not been elucidated, but the components responsible may act directly by neutralizing the azelaic acid or indirectly by affecting the physiological state of the micro-organisms.

Substantial reductions in bacterial counts have been observed during clinical trials of topical azelaic acid (Bladon *et al.*, 1986). These reductions do not necessarily indicate bactericidal activity *in vivo* since depression of microbial growth rates could also cause a reduction in numbers due to the displacement of bacteria in the constant flow of sebum and sloughing of cells. Azelaic acid has been reported to alter several aspects of skin physiology (Breathnach, Nazzaro-Porro & Passi, 1984), and therefore it is possible that the apparent antimicrobial effect noted is an indirect one caused by an adverse change in the cutaneous environment.

Further investigations are necessary to determine the mechanism of action of azelaic acid *in vitro* and *in vivo* and whether consequent reductions in bacterial densities on the skin are important in the improvement in acne vulgaris.

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# Demonstration of a unique viral structure: the molluscum viral colony sac

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## SUMMARY

Using scanning electron microscopy to study molluscum contagiosum lesions, it has been possible to demonstrate a unique, well defined sac enclosing the virion colony of each infected keratinocyte. This confirms the presence of a structure demonstrated over 50 years ago by micro-dissection, but ignored since it is not seen on either light or transmission electron microscopy. It is postulated that this sac, of unknown origin, favours replication of the virions by providing a site that is both anatomically and immunologically privileged. Such relatively unimpeded growth of the virions results in an enlarged cell in which the cytoplasm and nucleus are compressed into a thin outer shell. In the aggregate, this produces a tumour of virus-packed cells rather than a tumour composed of virus-induced cell hyperplasia.

Nearly 50 years ago, it was shown by micro-dissection that viral colonization in molluscum contagiosum took place within a sac-like structure, totally distinct from the infected cell (van Rooyen, 1938). This remarkable observation was later disregarded by histologists (Mehregan, 1961), histochemists (Mescon, Gray & Moretti, 1954) and electron microscopists (Dourmashkin & Bernard, 1959; Banfield & Brindley, 1959; Carteaud, 1968), who saw no evidence of such a structure. Furthermore, the principle that viruses exist as free elementary bodies or colonies within the cell (Andrewes, Pereira & Wildy, 1978) discredited van Rooyen's study. We have looked for the molluscum sac using scanning and transmission electron microscopy.

## METHODS

Twenty-four separate molluscum contagiosum lesions curetted from 14 patients (aged 2–28 years), were fixed immediately in a 2·5% glutaraldehyde solution. After being rinsed thoroughly in Millonig's phosphate buffer, each specimen was cross-sectioned with a Gillette Super Blue® razor blade, post-fixed in 1% osmium tetroxide, rinsed again in buffer and dehydrated step wise with ethyl alcohol. The specimens for scanning electron microscopy (SEM) were then critical

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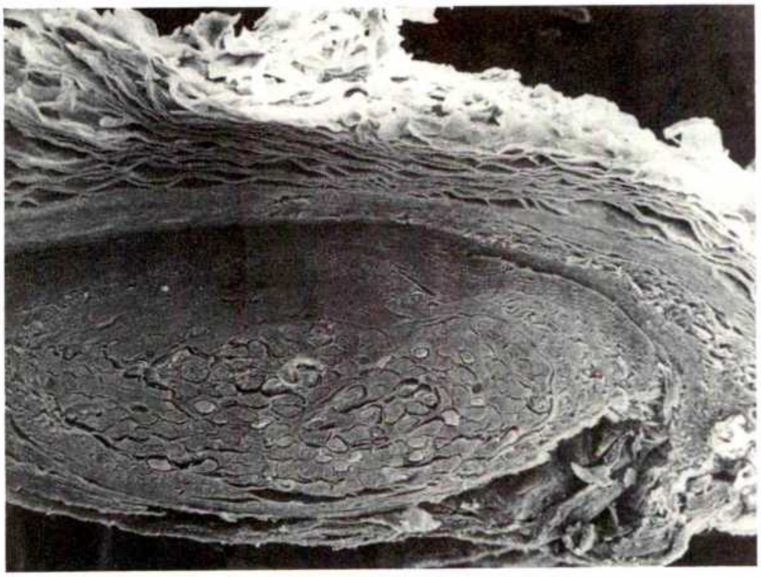

FIGURE 1. Scanning electron micrograph of cross section of molluscum contagiosum lesion in human epidermis. The ovoid structure is the tumour mass consisting of infected keratinocytes, and above it is the laminar mesh of stratum corneum. SEM (original × 550).

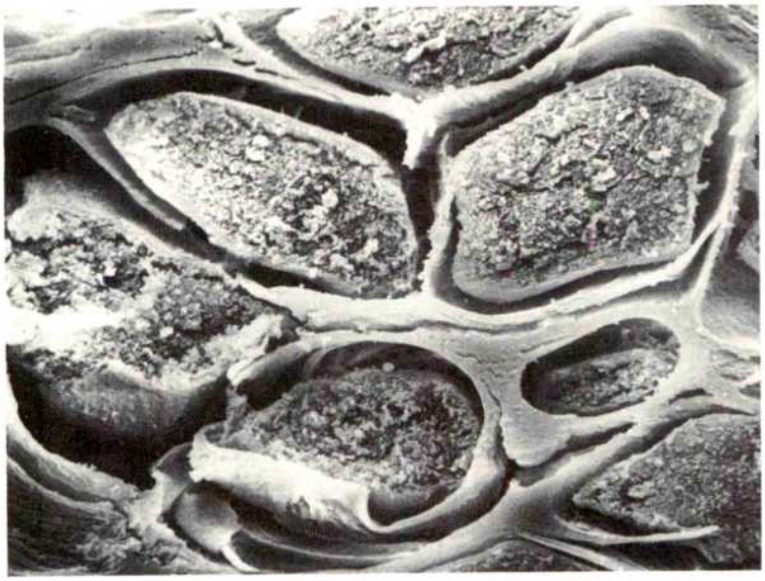

FIGURE 2. Intracytoplasmic molluscum colonies of packed virions. The colonies (molluscum bodies), are seen within membranous sacs which have pulled away from the highly condensed shell of the infected cells. At the upper left, interdigitating intercellular junctions between three keratinocytes can be seen. In the lower centre a translucent tongue of compressed keratinocyte cytoplasm is folded over a distinct membranous sac covering a colony of virions. SEM (original × 5500).

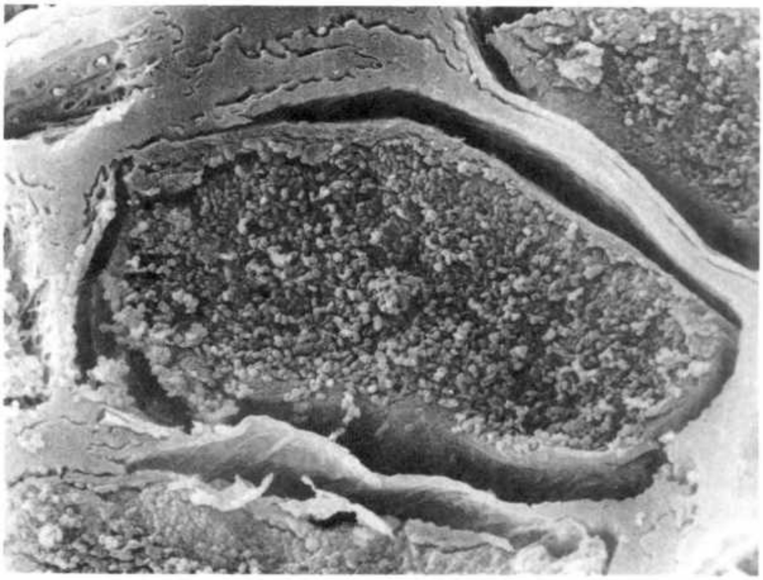

FIGURE 3. A keratinocyte with a thin amorphous shell of compressed cytoplasm and the colony of molluscum virions within a separate retracted membranous sac. SEM (original × 11 000).

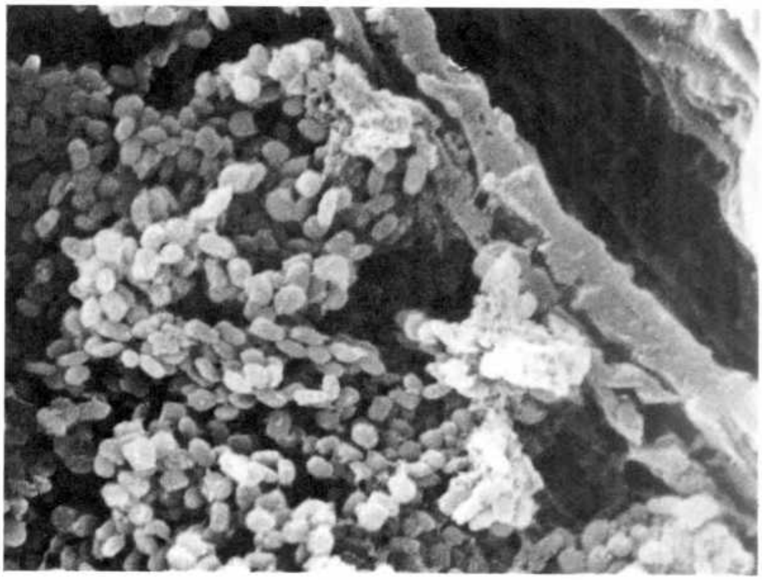

FIGURE 4. Discrete virions (0·30–0·35 μm in diameter) within a keratinocyte; the surface of adjacent keratinocytes can be seen at the upper right. SEM (original × 38 000).

point dried, positioned on a stub and sputter gold coated for 1·5 minutes at 15 mA. Examination was made with a JEOL scanning electron microscope (JSM-35, 25 kV, tilt angle 30°).

Comparative transmission electron microscope (TEM) studies were made on 10 selected specimens. Special treatment included snap freezing in liquid nitrogen before and also after glutaraldehyde fixation, as well as pre-soaking in xylene before glutaraldehyde fixation. Several specimens were embedded and studied after having been processed for SEM. Ruthenium red staining was employed in addition to regular staining with lead and uranyl acetate. Examination was made with a JEOL JEM-100 C transmission microscope.

## RESULTS

Scanning electron micrographs of the molluscum contagiosum lesion at increasing magnification show, in turn, the tumour mass of the distended infected keratinocytes (Fig. 1), colonies of virions within a distinct membranous sac displacing the normal cell contents (Figs 2 and 3) and myriads of the elongated virions themselves (Fig. 4). Transmission electron microscopy of selected well fixed specimens did not show the presence of an identifiable electron dense membrane surrounding the colony. However, the poorly fixed specimens (Fig. 5) and also the critical point dried gold coated specimens (initially prepared for SEM and then sectioned for TEM) did show on occasion a fine linear band surrounding the viral colonies (Fig. 6).

The limiting membrane surrounding the viral colony could not be seen using ruthenium red stain, nor was it evident on specimens which had been snap frozen in liquid nitrogen before or after fixation. Specimens pre-treated with xylene also failed to show the membrane.

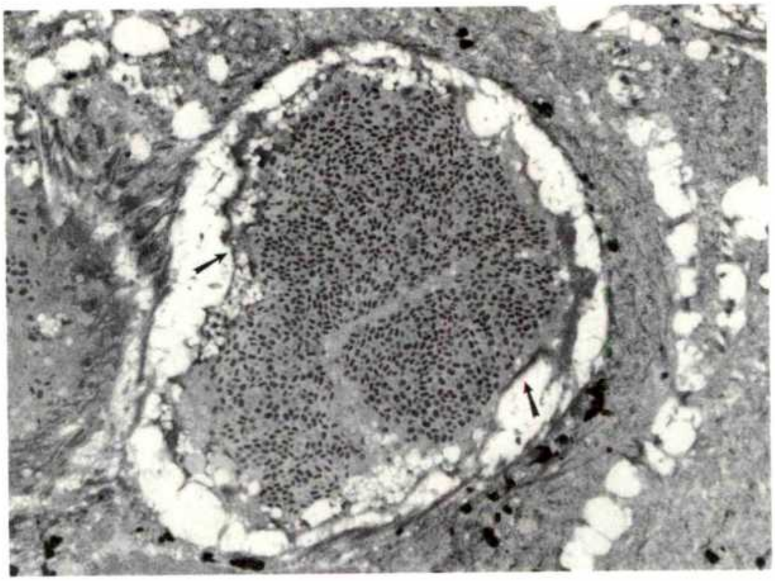

FIGURE 5. Transmission electron micrograph of molluscum contagiosum infected cell. Mature virions are dark ovoid bodies within the colony and immature virions are translucent forms at the periphery and in the trabeculae. A thin limiting membrane surrounds the virus colony (arrows) and has pulled away from the outer rim of condensed cytoplasm. The large dark masses within the cell cytoplasm are keratohyalin granules. At the extreme right can be seen space and desmosomes between this and the adjacent keratinocyte. TEM (original × 11 000).

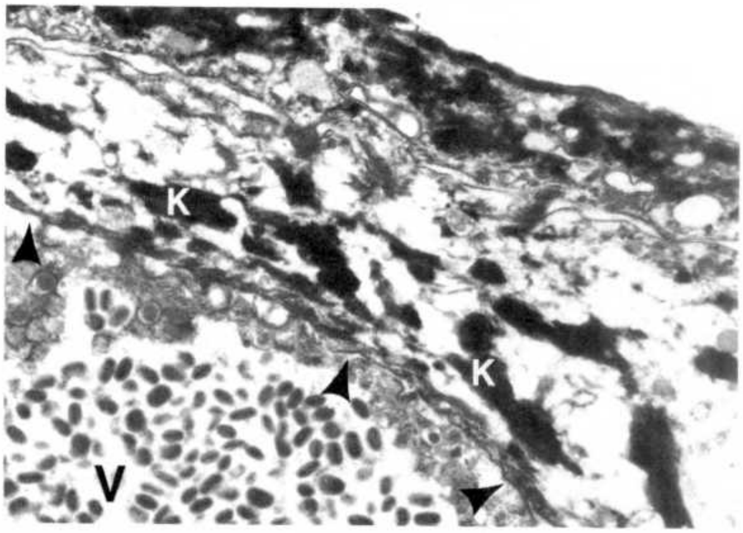

FIGURE 6. Higher power view showing section of infected cell with mature virions (V). The immature virions are in a fine granular matrix which is delimited by a thin electron dense membrane (arrows). Outside the membrane are irregular keratohyalin granules (K). Empty space at upper right is artefactual. TEM (original × 25 000).

## DISCUSSION

The molluscum virus is a large DNA pox virus, but its behaviour is anomalous (Brown, Nalley & Kraus, 1981). It does not form a 'pox' (necrotic lesions), but rather induces a benign tumour; it cannot be grown in tissue culture and it is not experimentally transmissible to animals (Dales & Pogo, 1981), although it is readily transferred from person to person.

The presence of a membranous sac surrounding the virion colony within the infected keratinocyte provides a morphological basis for some of these observations. The sac should afford protection from the lytic enzymes and organelles of the cell, grant immunological isolation, and allow virions to replicate to a degree not seen in other viral diseases. Thus, there is no cell necrosis, only a hypertrophied virus-filled cell in which the cytoplasm and nucleus have been compressed into a thin outer shell as seen in the SEM photographs. The cell is truly atrophic, both in function and in intracellular cytoplasm, but hypertrophied in size. Thus, the tumour is not the product of cell hyperplasia as are those induced by the papova viruses, but rather of a rare form of intracellular viral colonization.

Molluscum contagiosum occurs not only in man, but also in horses (Rahaley & Mueler, 1983), kangaroos (Bagnall & Wilson, 1974) and chimpanzees (Douglas *et al.*, 1967). Yet, there has been a failure to grow the virus and transmit the disease experimentally. This may reflect either the failure of investigators to use free virions, or the inability of the virus to enter the cell and form or induce a protective sac around its colonizing particles.

Although the molluscum colony sac is a unique structure in human viral infections, other examples are known in general virology. The X14 latent rat virus (Mayor, 1973) and the fowl-pox virus of turkey origin show membrane-bound inclusions (Cheville, 1966), and the virus causing polyhedrosis in insects secretes a polyhedral protein matrix which encases the colony

(Smith, 1973). This structure survives the death of the infected larva, and liberates the virions when eaten by other larvae.

Only one other observer has reported on scanning electron microscopy of molluscum contagiosum (Carteaud, 1968, 1969), and he failed to note any mebranous sac. We were able to demonstrate the sac unequivocally only by using critical point dried, transected specimens for scanning electron microscopy. However, we could produce, by freeze-thawing (Fig. 5) and by critical point drying (Fig. 6), evidence suggestive of the same membrane on transmission electron microscopy. Although we realize that what was seen was artefactual in both cases, the SEM demonstration of the membrane is convincing and significant. The superiority of scanning electron microscopy over transmission electron microscopy for the demonstration of membranes which are not electron dense on staining has been shown with the basement membrane. Heathcote and Grant (1981) showed the basement membrane as a real, three-dimensional sheet when viewed with the scanning electron microscope, whereas the same membrane is a very poorly defined structure in cross-sections viewed by transmission electron microscopy.

The source of the membrane remains unknown, although van Rooyen (1938) suggested that it was a mucopolysaccharide elaborated by the virus itself. Later, Low (1946) suggested that the sac could be a glue-like material of virion origin but which formed a coagulum or membrane at the interface with the cell cytoplasm. In support of this view, van Rooyen (1938) had observed that the virions were embedded in a jelly-like substance within the sac which he had microdissected out of the infected cell.

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# Excision of skin tumours without wound closure

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## SUMMARY

Sixty-two patients with 67 large or poorly defined skin tumours predominantly on the head and neck (58 basal cell carcinomas) were treated by excision of the lesion and allowing the defect created to heal by second intention. Histological control of the adequacy of excision was monitored using routine vertical sections of formalin-fixed tissue. Further re-excisions were performed in 17 patients in whom tumour extended up to or within 1 high power field (approximately 0·44 mm) of the excision margin. The formalin-fixed specimens ranged from 6–60 (mean 21) mm in diameter and 2–12 (mean 5) mm in depth. After one excision the time to complete re-epithelialization was directly proportional to the surface area ($r = 0{\cdot}73$) and ranged from 13 to 60 days (mean 33 days). Measurements of the movements of fixed reference points tattooed at the wound edges in six patients showed that movement of surrounding tissue into the defect accounted for 39–62% (mean 45%) of the reduction in surface area of the defect during healing. Post-operative complications were rare and the cosmetic results were considered good or excellent in 48 patients, fair in nine and poor, i.e. requiring corrective surgery, in three patients. Poor results were due to distortions of free margins, e.g. lower eyelid and nasal margin. The major benefit of this technique is the ease with which further excisions can be performed when histologically indicated. In selected areas this simple and quick technique avoids the need for repair with full thickness grafts or flaps, and the defect shrinkage and excellent contour and colour matching produce cosmetic results superior to those of split skin grafts or following radiotherapy.

The most important consideration in the treatment of malignant skin tumours is the complete removal or destruction of the tumour. Tumours may spread beyond the visible margins and an inexperienced surgeon preoccupied with closing the wound may reduce the margin of excision to facilitate the repair, thus leaving residual tumour. The immediate repair of larger defects with full thickness grafts or flaps before histological confirmation of complete tumour removal can result in recurrences which may be advanced before becoming clinically apparent.

Excision of skin tumours without closure is a simple, quick out-patient procedure easily performed under local anaesthetic. The technique has the advantage that the site of any possible

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residual tumour can be judged from the histological specimens and further excisions made, if histologically indicated, without compromising a previous repair. The disadvantages are the prolonged healing time and occasionally poor cosmetic results. This technique has long been championed by Mohs (1958) and others (Tromovitch & Stegman, 1974; Goldwyn & Rueckert, 1977; Zitelli, 1983) who used frozen tissue horizontal sections for microscopic control. This technique provides the most certain evidence of complete tumour excision but processing these histological sections is a time-consuming and highly skilled procedure that is not readily available in most general hospitals. For the past 3 years we have excised large tumours and those with indistinct margins and have allowed the wounds created to heal by second intention but have used routine, fixed tissue, vertical sections for histological control. We report here our results in 62 patients, including an investigation of the relationship of wound size to healing time and the role of wound contraction.

## METHODS

### *Patients*

Between January 1982 and April 1984, 219 patients with skin malignancies were seen in whom surgical excision of the skin tumour was considered the appropriate form of treatment. In 62 of these patients (27 female, 35 male, age 21–87 years, mean 63 years) surgical excision was performed and the wounds created allowed to heal by second intention. This technique was used when the defect created by tumour excision was too large for closure by primary suture, or the tumour had an indistinct margin. Fifty-seven were treated as out-patients and five were inpatients.

### *Tumours*

The 67 tumours included 58 basal cell carcinomas (BCC), four squamous cell carcinomas (SCC), three malignant melanomas and two keratoacanthomas. Twelve of the BCCs were recurrent tumours, five following previous excision, four after curettage, two after radiotherapy and one after cryotherapy. Clinically, 48 of the BCCs were classified as nodulo-ulcerative, eight morphoeic, one pigmented and one superficial spreading. Sixty of the lesions were on the head and neck and involved the following sites: scalp 1, forehead 7, temple 9, medial canthus 5, nosetip 7, nose bridge and side 7, nasolabial fold 11, lower eyelid 2, cheek 11, on and around the ear 4, back 2 and hand 1.

### *Excision technique*

For BCCs a margin 2–3 mm clear of visible and palpable tumour was marked and under local anaesthesia (2% lignocaine with 1 : 200 000 adrenalin) the entire area excised, including the full thickness of the dermis and a depth of subcutaneous fat and sometimes muscle judged clinically appropriate. At certain sites, e.g. temple or pre-auricular area, the subcutaneous tissues were lifted from underlying deep fascia by injecting 10–20 ml of normal saline immediately prior to surgery. A margin of at least 10 mm was used for malignant melanoma and 5 mm for SCC. The wound was dressed with 10% povidone-iodine ointment applied to a non-adherent dry absorbent dressing (Melonin®, Smith & Nephew, Welwyn Garden City, Herts, U.K.) and a pressure bandage applied. Dressings were changed twice a week for the first 2 weeks and weekly thereafter. Once a crust had formed, the area was left open and painted daily with gentian violet. Care was taken not to allow exposed periosteum or perichondrium to dessicate.

A marking suture was placed at one pole to orientate the specimen which was then fixed whole in 10% formol saline. After fixation for about 24 h, the diameters of the fixed specimens were measured and recorded by the histopathologist. Vertical sections were taken through the two diameters at right angles. Further sections were prepared if indicated. Re-excisions were performed if tumour extended to the edge of the specimen or to within 1 high power field (0·4 mm).

### *Measurement of wound healing and contraction*

Measurements of diameter and depth of the formalin-fixed excised specimen and the time to heal, taken as the time of complete wound epithelialization, were available in 37 patients who required only a single excision. In six patients, two each with tumours on the cheek, nose and temple, measurements of the rate and amount of wound contraction were obtained. Using a fine needle, specks of indian ink were tattooed into the skin 2–3 mm beyond the wound margin on two lines at right angles corresponding to the maximum and minimum diameters of the defect. The distances between these marks were measured serially during and after wound healing. From these measurements the change in diameter, surface area and perimeter of the defect, and later the re-epithelialized scar, were calculated.

## RESULTS

### *Size of excision*

The 67 specimens removed were oval or round and the fixed tissue maximum diameter ranged from 6–60 mm (mean 21 mm) with a mean depth of excision of 5 mm (range 2–12 mm).

### *Repeat excisions*

After initial excision, histological examination revealed tumour extending to the excision margin in 10 patients (all BCCs) and to within 1 high power field in seven patients. Re-excisions were performed in all 17 patients, two of whom required two and three re-excisions respectively before histological evidence of complete tumour removal. Residual tumour in the re-excision

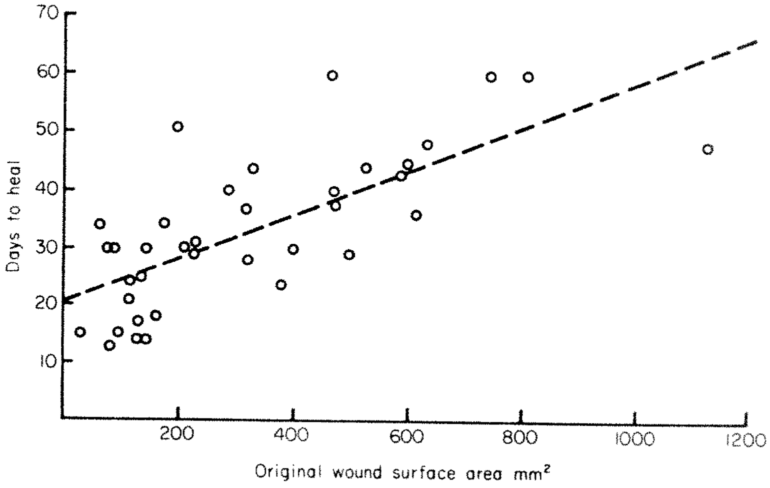

FIGURE 1. Time to heal after one excision in 37 patients related to original wound size. $r = 0{\cdot}73$, $P < 0{\cdot}001$. Surface area (SA) calculated for eliptical excisions with diameters $d_1$ and $d_2$ according to the formula $SA = d_1 \times d_2 \times \pi/4$.

specimens was found in four of the 10 patients (40%) in whom tumour had been identified previously extending to the excision margins. Tumour was not identified in re-excised specimens from the seven patients in whom tumour had extended to within 1 high power field of the previous excision margin.

### *Wound healing and contraction*

Healing time in 37 patients who required only one excision ranged from 13–60 days (mean 33 days) and was directly proportional to the size of the excision. The maximum diameter of these excised specimens ranged from 6–42 mm (mean 22 mm). We examined the relationship between the smallest diameter, the surface area and the volume of tissue excised and healing time. The highest correlation was with surface area ($r = 0{\cdot}73, P < 0{\cdot}001$, Fig. 1). Slightly lower correlations were obtained with smallest diameter ($r = 0{\cdot}67; P < 0{\cdot}001$) and volume of excised tissue ($r = 0{\cdot}62; P < 0{\cdot}001$). The effect of excision depth was studied by analysing the depth of the fixed specimens examined in relation to the data shown in Figure 1. Points above the line represent wounds which healed more slowly than average and these wounds had a mean excision depth (± SEM) of $4{\cdot}66 \pm 0{\cdot}65$ mm. Points below the line represent wounds that healed more rapidly than average and these had a mean excision depth of $4{\cdot}50 \pm 0{\cdot}42$ mm. This difference was not statistically significant ($P > 0{\cdot}05$, Student's $t$-test).

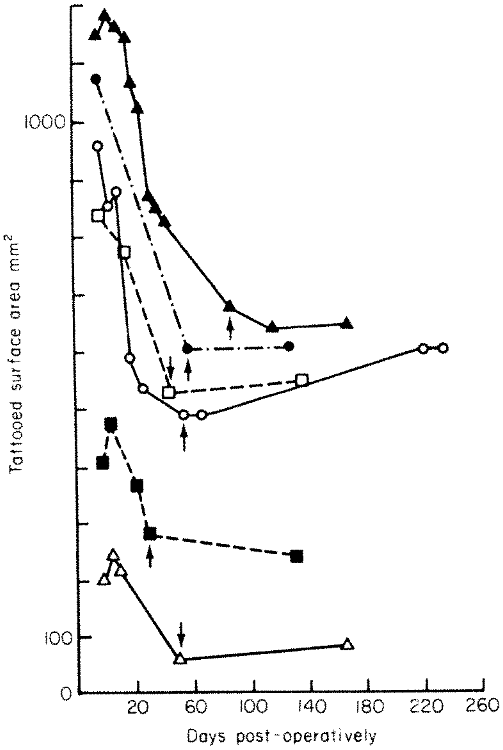

FIGURE 2. Change in tattooed wound surface area with time in six patients. One patient (△) required a further excision to obtain histological clearance. Arrows indicate time when wound epithelialization was complete. Sites: temple ● and ▲; nose ■ and △; cheek ○ and □.

Measurements of wound contraction rate were made in six patients. Contraction rate was proportional to the change in diameter and perimeter of the tattooed area but correlated best with surface area. A similar pattern of response was seen in each patient (Fig. 2). During the first week, the tattooed surface area increased slightly in three of four patients measured, followed by a rapid decrease in all 6 patients to the point when full epithelialization occurred at an average of 55 days (range 30–90 days). In three patients there was a subsequent slight increase in the surface area of the scar. After an average 167 days (range 130–238 days) the final scars had a surface area 55% (range 39–62%) of the original size.

*Cosmetic results*

Patients were graded by the operator as an excellent cosmetic result in 15, good in 33, fair in 9 and poor, requiring corrective surgery, in 3. Examples are shown in Figures 3, 4 and 5. In two other patients complete excision of the BCC resulted in the creation of a full thickness defect on the nose. In one 79-year-old man with severe cardiac disease this was covered by a prosthesis, and in the other a full thickness skin flap was rotated to cover the defect. In general, better results were obtained at the medial canthus, nasolabial fold and on the ear. On the nose, generally

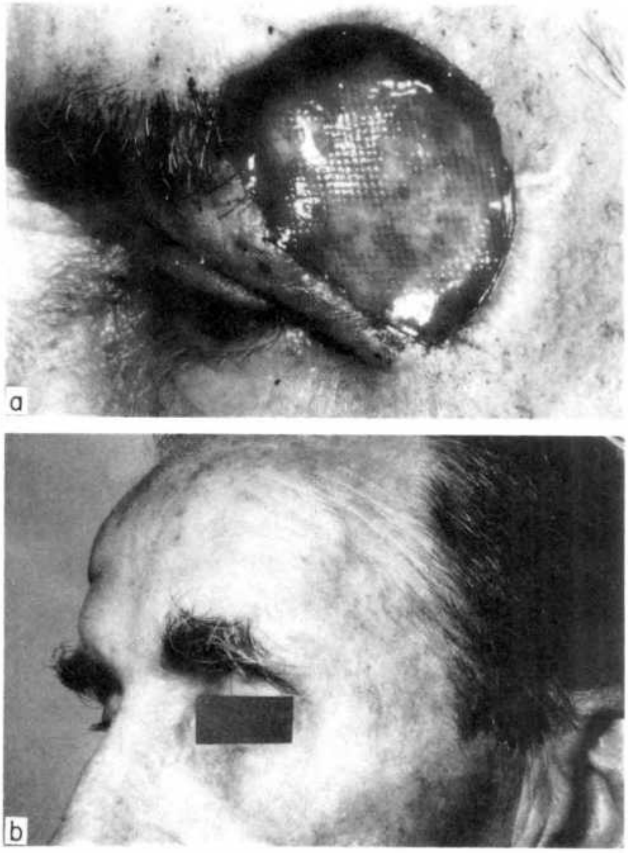

FIGURE 3. Example of an excellent result. (a) Wound appearance one week after two excisions of a morphoeic BCC, down to periosteum, from the left eyebrow of a 62-year-old man. (b) Appearance 20 months later.

poorer results were obtained on the tip, whereas wounds on the bridge and side resulted in satisfactory scars. During the first 2–3 months, scars were often nodular and red but the appearance continued to improve for up to a year, becoming flat with a good contour and colour match blending into the surrounding skin. Excision of large BCCs from the temple resulted in some weakness of the frontalis muscle in three patients due to division of branches of the facial nerve. Gradual improvement in muscle function occurred in two of the patients over a follow-up period of 2 years and the cosmetic disability was minimal in all three. Poor results requiring corrective surgery were obtained in two patients who had excisions from the lower eyelid resulting in an ectropion and a third who had a BCC excised from the temple causing distortion of the eyebrow. Excellent cosmetic correction was obtained in all three with use of full-thickness grafts.

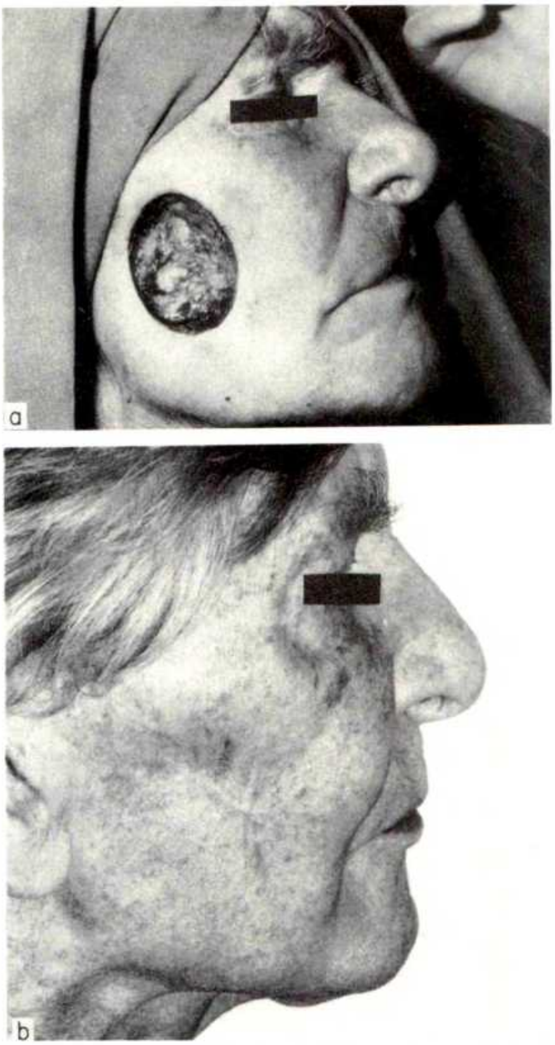

FIGURE 4. Example of a good result. (a) Appearance immediately after excision of a melanoma from the cheek of an 84-year-old man. (b) Appearance 13 months later.

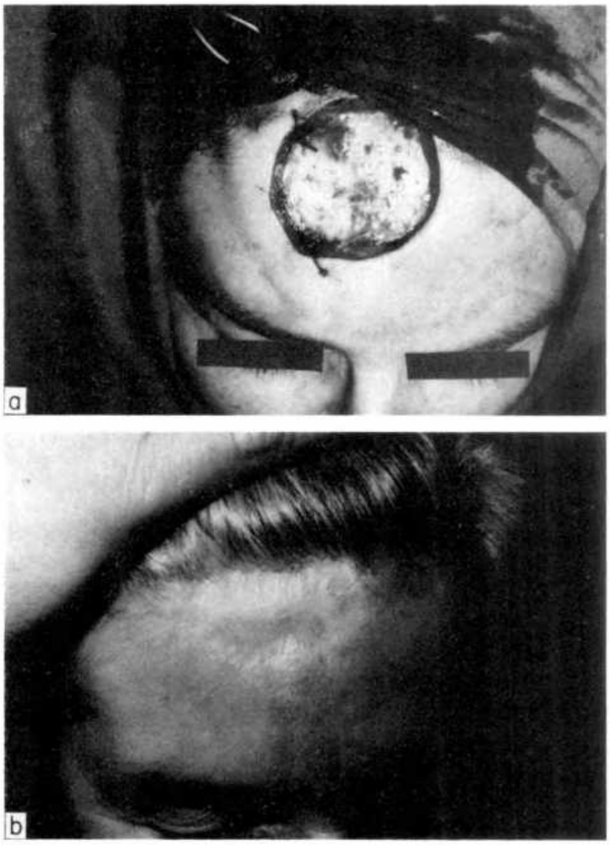

FIGURE 5. Example of a fair result. (a) Wound appearance immediately after excision of a morphoeic BCC from the forehead of a 47-year-old woman. (b) Appearance 18 months later.

*Post-operative complications*

During the first 7–10 days post-operatively, the wounds were usually covered with a yellow exudate. This was not necessarily indicative of bacterial infection or colonization. *Staphylococcus aureus* was isolated from 12 wounds but in only two was there swelling, pain and redness of the surrounding skin indicating significant infection which responded promptly to antibiotics. One patient returned within 12 h with oozing from the wound surface after the pressure bandage had slipped. Otherwise, bleeding post-operatively was not an important problem. After initial discomfort lasting 24–48 h, the wounds were remarkably pain-free.

*Follow-up*

Sixty-one patients have been followed up for an average of 23 months (range 10–40 months). One has been lost to follow-up. There have been no local tumour recurrences, but one patient with an SCC on the ear developed a cervical lymph node metastasis.

## DISCUSSION

The technique of excision without closure has many advantages for the excision of skin malignancies, particularly BCCs. These include simple re-excision if histologically indicated, simple surgical technique, short operating time, and good to excellent cosmetic results in the majority of patients.

The most important advantage of this non-closure technique is the ease with which further excisions can be made if indicated histologically. Serial excision is particularly suited to tumours which spread only by local extension, such as BCCs, which formed 86% of the tumours in this series. The extent to which some BCCs infiltrate laterally beyond the visible tumour margins is commonly under-estimated, leading to inadequate excision. The importance of obtaining histological evidence of complete tumour removal before covering such defects with flaps or grafts has been emphasized previously (Gooding, White & Yatshuhashi, 1956; Koplin & Zarem, 1980). The technique of Mohs' surgery (Mohs, 1958), in which the entire lateral and deep wound margins are examined microscopically for residual tumour, offers the most certain way of ensuring complete tumour removal. However, this technique is time-consuming and requires skilled technical assistance which is not generally available in the U.K. The histological techniques used in this series on formalin-fixed specimens give an incomplete view of the excision margins but have the advantage of being routine in any histopathology laboratory. Only long-term studies will establish the cure rates of this technique, but there have been no local tumour recurrences to date in the present series.

Post-operative problems were few. Haemorrhage and infection were infrequent despite colonization of almost 20% of wounds with *Staphylococcus aureus*. The absence of clinical infection may be due to the absence of foreign suture material in the tissues (Elek & Conen, 1957). Despite the creation of wounds up to 6 cm in diameter, pain was always mild and limited to the first 3 post-operative days. No patient required analgesia stronger than paracetamol and many used no analgesia at all.

After one excision the healing time of the wounds created was linearly related to the wound surface area. The correlation with wound volume was not as high and, within the limits studied, wound depth (2–12 mm, mean 4·5 mm) appeared to be of little importance in determining healing time. Early in our observations it became apparent that wound contraction was a major contributing factor to the general excellence of the final cosmetic result. In hairy mammals full thickness wounds heal principally by wound contraction due to the presence of a panniculus carnosus with little subcutaneous fat or attachment to deep fascia (Edwards & Dunphy, 1958; McGrath & Simon, 1983). The present study demonstrated that contraction accounted for 45% of the wound healing process in the six patients in whom this was measured. This factor has been thought previously to play only a minor role in wound healing in man (Billingham & Medawar, 1955).

The disadvantages of the technique are the prolonged healing time compared with immediate repair procedures, and the occasional poor cosmetic result. Wounds in our patients healed after an average of 33 days, range 13–60 days. After full epithelialization, the wounds continued to mature for 6–12 months. Initially, a small hypertrophic pink scar developed. This was followed by flattening of the scar and a gradual return of normal skin colour with a margin that blended with the surrounding skin. It is important to be familiar with these phases of wound healing to be able to distinguish them from tumour recurrence. Cosmetic results were only fair or poor in 12 of our patients. Three factors contributed to this. Firstly the size of the wound; in general wounds greater than 25 mm in diameter produced a less favourable result than small excisions. Secondly, the site of the excision; wounds adjacent to mobile skin margins such as the eyelid, lip or external nares, may produce distortion of the free margin. Finally, the age of the patient; both age and actinic damage reduce the likelihood of hypertrophic scarring. Our patients were elderly, mean age 63 years, and the cosmetic results which we have observed may be less favourable in younger patients.

Conventional surgical techniques may produce less post-operative morbidity and a more predictable and superior cosmetic result with minimal scar formation. Rapid margin control can be achieved with frozen sections, but this adds considerably to operating time and to the cost of the procedure and is unsuitable for routine use. Conventional histological techniques for margin control require that a closure procedure is delayed. There is no reason why such a delayed closure should not be made after complete tumour removal using the technique which we have described. However, after it has been explained to them that the wounds will heal satisfactorily, if more slowly, without formal repair, we have found that many patients prefer to avoid an additional surgical procedure. In our experience the cosmetic results achieved by second intention healing are generally superior to split skin grafts or following single exposure radiotherapy, and the histological control adds the advantage of ensuring the adequacy of the excision. The avoidance of formal closure procedure also has a major cost implication, particularly if the repair would have otherwise required hospital admission and general anaesthesia; thus it may be useful in parts of the world where resources are limited or a plastic surgeon is not available.

We believe that this technique is an effective method for managing many patients with malignant skin tumours. It is particularly useful for recurrent tumours or those with indistinct margins since it enables the easy re-excision of residual tumour. Provided the operator understands the limitations posed by the site of the tumour and the age of the patient, a generally good cosmetic result can be achieved.

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# No association between malignant melanoma and acne or psoriasis: results from the Western Canada Melanoma Study

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## SUMMARY

In a study of 651 patients with cutaneous malignant melanoma and matched controls, no association was found with a history of acne vulgaris or psoriasis. Examination of the severity, age at onset and treatment for acne or psoriasis, and analysis by age, sex and site distribution of melanoma, revealed no evidence of any association.

Individuals with psoriasis have been reported to be at increased risk of melanoma (Beral *et al.*, 1983) and of other skin cancers (Halprin, Comerford & Taylor, 1982). In a follow-up study reported by Stern *et al.* (1979), there was a suggestion of an excess of cutaneous cancers reported in psoriasis patients treated with 8-methoxypsoralen phototherapy. However, Pembroke *et al.* (1979) suggest that any excess detected might easily be due to prior radiation or arsenic treatments in long-term psoriasis patients. Alderson and Clarke (1983) examined the possible relationship between a prior history of psoriasis and skin cancer in Scotland, using medical record linkage techniques. The authors acknowledge that hospital discharge summaries might not be ideal diagnosis sources for psoriasis patients, and also that being under a physician's care for psoriasis may make detection of a subsequent skin cancer more likely. Although they observed slightly more skin cancers in the psoriasis group than expected, the excess was not statistically significant.

Beral *et al.* (1983) found a less frequent prior history of acne vulgaris in melanoma patients than in controls. They suggested that this might be due to increased sebum production in acne, as ultraviolet radiation is absorbed by skin surface lipids (Beadle & Burton, 1981). Recently, there has been some concern over the use of benzoyl peroxide in the treatment of acne (Veitch, 1985; Burton, 1985). This compound has been found to be a tumour promotor in mice

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(Grindhart, Srinivas & Colburn, 1985; Slaga *et al.*, 1981), and was implicated as a possible carcinogen in a study of malignant melanoma in chemists (Wright, Peters & Mack, 1983).

We have therefore examined the data from the Western Canada Melanoma Study with regard to history of both acne and psoriasis and their treatment.

## METHODS

The methodology of the Western Canada Melanoma Study has been described previously (Elwood *et al.*, 1984; 1985); detailed home interviews were sought with all residents of four western provinces of Canada who had a newly diagnosed cutaneous malignant melanoma between 1979 and 1981, and with age- and sex-matched controls drawn randomly from the source populations. Eighty-three per cent of eligible melanoma patients were interviewed along with matched controls. Pathology slides were reviewed and classified centrally. The present analysis concerns 651 patients, of whom 415 had superficial spreading melanoma, 128 nodular melanoma, 52 unclassified or borderline melanoma, and 56 lentigo maligna melanoma. Each subject was asked 'Do you have or have you had acne (that is, multiple pimples, and blackheads, or scars, mainly on the face)?', with supplementary questions about the age at which acne was first noticed, whether it was only a minor problem, was quite noticeable but not requiring treatment by a physician, or whether it was severe enough to cause considerable embarrassment or worry and to require treatment from a physician. Questions were also asked about treatments given and the names of drugs used. A similar question was asked regarding psoriasis.

Analyses used cross tabulations and chi square statistics, or where numbers were small, Fisher's exact test. For the major variables of history and severity of acne and psoriasis, matched pair analyses were performed, giving results very similar to the unmatched analyses. Further analyses were done for melanoma subdivided by sex (387 female, 264 male patients), by subtype (as above), by body site (head and neck, 126; trunk, 194; upper limb, 136; lower limb, 183; others, 12), and by age ($<34$ years, 184; 35–49 years, 282; 50 years and over 185); for these analyses the relevant subsets of patients were compared to their age- and sex-matched controls.

## RESULTS

A history of acne was reported by 157 (24·1%) melanoma cases and 151 (23·2%) of the controls, giving a relative risk of 1·05, 95% confidence limits 0·82–1·36, (Table 1). Six percent of cases and 6·9% of controls reported having had severe acne, and 9·1% of each group had acne which had started before the age of 14. Very similar proportions of cases and controls had used treatments such as special soaps or cosmetics, non-prescription or prescription topical applications, oral medications, X-ray or ultraviolet light. Of 48 cases and 56 controls who reported having used drugs to treat acne, 37 cases and 38 controls named two or more specific items. These were most frequently soaps and sulphur or resorsinol compounds; preparations containing benzoyl peroxide were mentioned by very few subjects, and there were no differences in the types of drugs used by cases and by controls.

Psoriasis was reported by 41 (6·3%) cases and 34 (5·2%) controls, giving a relative risk of 1·22 with 95% confidence limits of 0·76–1·94. There were no major differences between the cases and controls in the severity of psoriasis, the age at which it was first noticed, or the treatments given. The most frequently used treatments were prescribed topical ointments. Drugs were used by 27 cases and 23 controls, but named drugs were identified by only nine cases and six controls, the most frequently mentioned being steroids, and no differences were apparent between cases and controls.

TABLE 1 Frequency of histories of acne vulgaris and of psoriasis in 651 patients with cutaneous malignant melanoma and 651 age- and sex-matched control subjects

| | Acne vulgaris | | Psoriasis | |
|---|---|---|---|---|
| | Cases | Controls | Cases | Controls |
| Positive history | 157 | 151 | 41 | 34 |
| Severity: | | | | |
| mild | 77 | 73 | 16 | 10 |
| moderate | 41 | 32 | 3 | 5 |
| severe | 39 | 45 | 25 | 18 |
| Onset: | | | | |
| < 13 years (acne) | 59 | 60 | | |
| < 25 years (psoriasis) | | | 16 | 12 |
| 14+ years (acne) | 95 | 87 | | |
| 26+ years (psoriasis) | | | 17 | 16 |
| unknown | 3 | 4 | 8 | 6 |
| Treatment: | | | | |
| soaps | 27 | 35 | 5 | 7 |
| non-prescribed creams | 31 | 29 | 7 | 2 |
| prescribed creams | 23 | 28 | 19 | 16 |
| oral medication | 15 | 14 | 0 | 6 |
| X-rays | 3 | 4 | 0 | 1 |
| UV light | 9 | 11 | 3 | 2 |
| surgery | 1 | 1 | 0 | 0 |

None of the differences between cases and controls are significant at the 0 05 level, using chi squared tests or Fisher's exact test.

Analyses were performed separately for males and females, for the different types of melanoma, and for melanoma on different body sites. There were no differences between cases and controls apparent on any of these analyses. For subjects with melanoma of the head and neck region and their matched controls, the prevalence of reported acne was 18·5% in cases and 16·9% in controls.

## DISCUSSION

This is the largest analytical study of malignant melanoma currently available and the results are based on extensive standardized interviews carried out by trained interviewers in the subjects' homes on 651 patients with newly diagnosed cutaneous melanoma, and an equal number of age- and sex-matched control subjects drawn randomly from the same population. The data are therefore likely to represent the best current information, given the limitation that the histories of acne and psoriasis are based on the patient's own recall and no physical examination was carried out. The medical records of the melanoma patients were also scrutinized, but these were found to give much less information on other diseases than did the interviews, and no comparable information was available for the controls.

The results showed that the frequencies of acne and psoriasis reported by melanoma patients and by controls were very similar, and argue strongly against there being any substantial increase or decrease in melanoma risk associated with these conditions, at least as they occur and are treated in Western Canada. As would be expected, acne was commonly reported, but most often as a mild disease not requiring specific therapy, and specific treatments such as ultraviolet therapy were used in only a few individuals. Preparations containing benzoyl peroxide were not used extensively in these patients, and therefore we can provide no definitive information on whether any risk might accrue to subjects who do use such preparations.

Our results do not confirm those of Beral *et al.* (1983), who compared 287 women with malignant melanoma, aged 18–54 years, with 574 controls, and found a significant positive association between melanoma and psoriasis, and a significant negative association between melanoma and acne. The prevalence of psoriasis in that study was 1·1% ($n=6$) in controls and 3·2% ($n=9$) in cases; for women of the same age range in our study the prevalence was 6·8% ($n=16$) in controls and 5·7% ($n=13$) in cases. Prevalence rates for acne were not given. The differences between these two studies may simply be due to chance variation or to differences in the interview questions. Berel *et al.* (1983) collected no information on treatment. It remains possible, but seems unlikely, that the treatment policies for these diseases might be very different in Australian women and provide an explanation for the difference in results.

## ACKNOWLEDGMENT

We thank Dr J.L.Burton for suggesting this question, and acknowledge the assistance of other investigators and consultants in the Western Canada Melanoma Study, which was supported by the National Cancer Institute of Canada, Health and Welfare Canada (6610-1203-53), and the Alberta Heritage Trust Funds.

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# Low-dose etretinate in the maintenance of remission of palmoplantar pustular psoriasis

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## SUMMARY

Twenty patients with palmoplantar pustular psoriasis were treated initially for 4 weeks with 70 mg etretinate daily. This led to clinical improvement and a significant fall in pustule count. The patients were then allocated randomly to 30 mg etretinate daily or placebo for a further 12 weeks. There was a rapid deterioration in the clinical condition and a rise in pustule count in the placebo group. The etretinate-treated group still showed clinical improvement and a significantly lower pustule count after 12 weeks. Clinical side-effects were few and adverse effects on liver function and serum lipids were not found.

Etretinate has been used in the treatment of psoriasis (Orfanos, 1980) but its precise role remains unclear. It has, however, been shown to be particularly effective in inducing remission in palmoplantar pustular psoriasis (PPPP) (Foged *et al.*, 1981; Fredrickson & Peterson, 1979; Jansen, Hollman & Pajame, 1979), and used with PUVA produces a worthwhile reduction in the UVA dose required for clearance (Lawrence *et al.*, 1984). We have recently shown that a dose of 1 mg/kg body weight/day led to a significant improvement in the condition, and in about half the cases to complete clearance within 6–10 weeks (White, Marks & Shuster, 1985). Unfortunately, we subsequently found relapse after stopping etretinate to be common and rapid. The cutaneous side-effects, the risk of altered lipid metabolism (Gerber & Erdman, 1982), impaired liver function (Foged *et al.*, 1984) and possible bone changes (Gerber *et al.*, 1984), as well as its teratogenic effect, make etretinate far from ideal for prolonged use at this dosage for a chronic disease.

We have therefore tested the effectiveness of a lower dose of etretinate in maintaining remission once this had been induced by an initial 4-week course of 70 mg etretinate daily. The 4-week period was chosen on the basis of our previous trial in which any improvement that occurred was usually apparent between the third and sixth week of treatment. We were not necessarily aiming for complete clearance in this time as the majority of patients are willing to

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accept an improvement short of this as worthwhile. As there is so much variation in absorption of etretinate between individuals we decided to use the same dose in each patient rather than calculate it on a weight basis.

## METHODS

### *Patients*

Twenty patients (18 female and 2 male) with PPPP, attending the Skin Department at the Royal Victoria Infirmary, Newcastle, took part in the study. Only cases in which the diagnosis was not in doubt were included. The diagnosis was made clinically, as in our previous study, on the basis of well demarcated areas of erythema and scaling and the presence of sterile yellow pustules and brown dried up pustules on the palms or soles, or both, at the beginning of the study. Six patients also had psoriasis elsewhere. Females of childbearing age were required to use adequate contraception and to continue this for 12 months after stopping treatment. No patient selected had impaired renal or hepatic function. All antipsoriatic treatment was stopped at least one month before the trial, but emulsifying ointment was used throughout. The patients had been attending hospital for PPPP for between 6 months and 12 years (mean 3·2 years) and had previously received other treatments, including topical corticosteroids, tar, anthralin and PUVA. No patient had previously received oral retinoids.

### *Treatment regimens*

The trial lasted 16 weeks. After an initial 4-week period of taking 70 mg etretinate per day, patients were allocated at random to one of two treatment regimens, receiving either 30 mg etretinate per day or identical placebo capsules. This was continued for a period of 12 weeks. Neither patient nor doctor knew which patients were allocated to which group.

The two groups proved well matched for age (mean ± SEM, 54·7 ± 3·5 years for etretinate and 49·8 ± 4·8 years for placebo), and duration of disease (mean 3·6 ± 1·0 years for etretinate and 2·6 ± 1·1 years for placebo). There was no significant difference between the weights of the two groups (range 47–102 kg, mean ± SEM 67·2 ± 5 kg for etretinate, and range 53–95 kg, mean 74·4 ± 4·8 for placebo) ($P > 0{\cdot}1$, unpaired *t*-test). Consequently, the doses in the etretinate group were 0·68–1·48 mg/kg, mean 1·04 mg/kg during the 70 mg/day regimen and 0·2–0·63 mg/kg, mean 0·44 mg/kg during the 30 mg/day regimen; and in the placebo group 0·73–1·22 mg/kg, mean 0·94 mg/kg during the 70 mg/day regimen. These differences were not statistically significant.

### *Disease assessment*

The patients were examined prior to entry, and during the trial by one of two investigators (S.I.W. or L.P.). Before the trial, after the 4-week high-dose period and after 4, 8 and 12 weeks of the maintenance period, the number of fresh yellow pustules on the palms and soles was counted and the degree of scaling and erythema was scored as none (0), mild (1), moderate (2), or severe (3). At the end of the trial the patients were assessed overall as worse (0), no improvement (1), slight improvement (2), moderate improvement (3), good improvement (4), or clear (5).

### *Side-effects*

At each visit patients were questioned about the following: cheilitis, nose bleeds, conjunctivitis, headaches, pruritus, facial dermatitis, arthralgia, desquamation, malaise and alopecia.

### *Investigations*

Before the trial and at each subsequent visit, measurements were made of the serum aspartate transaminase, alanine transaminase, alkaline phosphatase, bilirubin, fasting cholesterol and triglycerides, and urea and electrolytes.

### *Statistics*

Results were analysed using Fisher's exact test, and Student's *t*-test.

## RESULTS

### *High-dose period*

In five patients all the pustules disappeared in the 4 weeks on 70 mg etretinate per day and the remaining patients, with one exception, showed a fall in pustule count (Table 1), giving a significant decrease in pustule counts for the group as a whole ($P < 0·01$; paired *t*-test). The patient whose pustule count rose had stopped his medication in the second week because of a sore mouth and dry skin, and subsequently continued to miss tablets frequently. He was withdrawn from subsequent analyses. The degree of scaling fell and the degree of erythema rose in all 20 patients during this period.

TABLE 1. Pustule counts in 20 patients after an initial course of 70 mg etretinate daily (high dose) followed by placebo or 30 mg etretinate daily (low dose).

| Patient no. | High-dose regimen | | | Low-dose regimen | | |
|---|---|---|---|---|---|---|
| | Pre-treatment | Week 4 | | Week 4 | Week 8 | Week 12 |
| 1 | 9 | 2 | *Placebo* | 19 | — | 3 |
| 2 | 52 | 4 | | 38 | 14 | 9 |
| 3 | 66 | 5 | | 127 | — | 66 |
| 4 | 103 | 10 | | 35 | 46 | 47 |
| 5 | 86 | 9 | | 70 | 154 | 56 |
| 6 | 134 | 1 | | 49 | 50 | 51 |
| 7 | 62 | 15 | | 20 | — | — |
| 8 | 7 | 0 | | 30 | 10 | 16 |
| 9 | 56 | 68 | | 26 | 3 | 36 |
| 10 | 22 | 2 | | 171 | 30 | 36 |
| mean ± SEM | 59·7 ± 13·4 | 11·6 ± 6·7 | | 58·5 ± 16·2 | 43·9 ± 19·5 | 35·6 ± 7·3 |
| 11 | 61 | 15 | *Etretinate* | — | — | — |
| 12 | 48 | 0 | | 0 | 13 | 1 |
| 13 | 98 | 21 | | — | 97 | 15 |
| 14 | 35 | 0 | | 0 | 0 | 0 |
| 15 | 14 | 0 | | 10 | 10 | 3 |
| 16 | 30 | 8 | | 15 | 10 | 29 |
| 17 | 11 | 0 | | 5 | 1 | 1 |
| 18 | 10 | 2 | | 5 | 1 | 1 |
| 19 | 89 | 9 | | 35 | 15 | 90 |
| 20 | 17 | 2 | | 11 | — | — |
| mean ± SEM | 41·3 ± 10·2 | 5·7 ± 2·3 | | 8·9 ± 4·0 | 18·4 ± 11·5 | 17·5 ± 11·0 |

### *Low-dosage maintenance period*

Ten patients were allocated to placebo and 10 to etretinate 30 mg per day. The initial pustule count was slightly lower in the etretinate group than in the placebo group. Four patients who were later allocated to etretinate and one in the placebo group were pustule free after the 4-week high-dose regimen.

Eight patients in the etretinate group completed the trial. Of the others, one had an episode of chest pain 2 weeks after starting the maintenance dose and was advised by her general practitioner to stop the tablets. There were no ECG changes or any rise in cardiac enzymes and there had been no rise in her fasting serum lipids. Data from this patient were not included in subsequent analyses. Another patient withdrew after 4 weeks on the low-dose regimen because she felt the treatment was not controlling her condition.

Eight patients in the placebo group completed the trial. The two who did not were one with poor compliance, already mentioned, and one who was withdrawn when she developed cellulitis of her leg after 4 weeks. This settled with antibiotics. Her data were not included in the analyses from that point.

### *Clinical assessment*

In the etretinate group at week 4 of the low-dose regimen there was no significant rise in the pustule counts compared with those at the end of the high-dose period. The counts were higher at weeks 8 and 12 than at week 4 but at week 12 the mean count was still significantly lower than the pre-treatment count ($P < 0{\cdot}05$; paired *t*-test) (Fig. 1). Although there was some individual variation, seven patients had a lower count at week 12 than before treatment. By contrast, the placebo group at week 4 had significantly increased pustule counts compared with those at the end of the high dose period ($P < 0{\cdot}02$; paired *t*-test). The counts were still higher at weeks 8 and 12. At week 12 the counts were lower than at the start of the trial, but this difference was not statistically significant. Again, there was some individual variation.

Scaling in the placebo group had reached pre-treatment levels by the eighth week of the maintenance period whereas in the etretinate group it was still reduced at the end of the twelfth

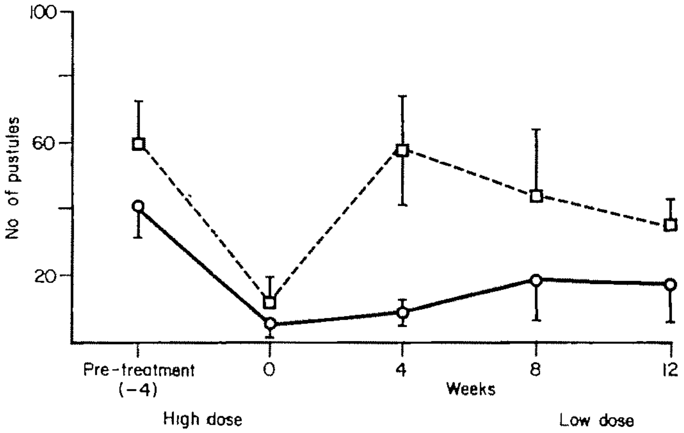

FIGURE 1. Pustule counts in 10 patients given etretinate (30 mg/day) —O—, and 10 patients given placebo, - - - □ - - -, after an initial 4-week high-dose regimen of etretinate (70 mg/day). Points are means ± SEM.

week, although this difference was not statistically significant. There was no clear difference in erythema between the two groups.

In overall clinical response no patient in either group was completely clear at the end of the trial. There was, nevertheless, a significant difference between the two groups; six out of eight patients on etretinate compared with one out of eight on placebo showed good or moderate improvement ($P < 0{\cdot}02$, Fisher's exact test). Two patients on etretinate and one on placebo were unchanged and the remaining six patients on placebo showed slight improvement.

### *Side-effects*

No patients withdrew from the trial because of the side-effects listed. The chest pain in one patient on etretinate and the cellulitis in one patient on placebo were considered to be unrelated to the treatment. A further patient on etretinate was obviously ill at her last attendance. She had thrombocytosis and central nervous system abnormalities attributable to infarcts. She was known to be suffering from essential thrombocytosis and cerebral vascular disease before the trial and it was not thought that her treatment in the trial had influenced these diseases at all. Although a clinical assessment of her psoriasis was made at her last attendance, she was admitted to hospital without the routine blood tests being done. One patient stopped treatment in the high-dosage period because of a sore mouth and dry skin, but later entered the maintenance part of the trial in which he proved to have been allocated to placebo. Seventeen of the 20 patients had cheilitis, three conjunctivitis, three pruritus, three facial dermatitis, and one patient reported nose bleeds during the high-dose part of the trial. During the low-dosage or placebo phase, four patients on etretinate continued to complain of cheilitis and two of facial dermatitis. In addition, one patient developed some hair loss. Two patients on placebo continued to complain of cheilitis throughout the 12 weeks of the low-dose period, but in most patients on placebo the side-effects had gone by the fourth week. We did not see the painful, red, glazed skin at the site of the healing lesions that others have described in people treated with etretinate.

### *Laboratory investigations*

The laboratory tests showed no abnormality, either initially, at the end of the 4-week high-dose period or at the end of the trial.

## DISCUSSION

The present study has confirmed our previous results (White, Marks & Shuster, 1985), which showed that a moderately high dose of etretinate substantially improved PPPP quite rapidly. On a dose of 70 mg daily for 4 weeks all of the patients showed a fall in pustule count and a decrease in scaling, although none cleared completely.

The main object of the present trial was to establish a suitable maintenance dose for this chronic condition, and the subsequent effect of 30 mg daily was investigated in a double-blind comparison with placebo. We believe this design of the study to be important, and the double-blind nature of the trial was not invalidated by the recognition of side-effects of etretinate, partly because all patients had had etretinate in the first part of the trial and there was a 'carry-over' effect.

In the maintenance part of the study there was a clear difference, especially in the early stages, between the two groups and, although this was less marked later, there was still significant benefit to be seen in the etretinate group at week 12 with respect to pustule count and scaling, and more patients in this group were regarded as generally clinically improved. Individual results showed no correlation between clinical response and dose expressed in mg/kg. It is difficult to know which of the four measurements we performed correlated best with clinical improvement, but patients often mentioned particularly the decrease in scaling as worthwhile. Our study suggests that the pustule count is a good indicator of disease activity. As in other studies with etretinate, measurement of erythema was not helpful in assessing improvement.

Our results agree with those of Lassus *et al.* (1983), who gave 40 patients 0·9–1·0 mg/kg/day etretinate until they had cleared. This varied from 4 weeks to a maximum of 16 weeks. Those who had cleared (26 of 40 patients) were then allocated randomly to placebo or etretinate 20–30 mg/day (0·14–0·38 mg/kg/day) and observed for 6 months. Seven of the 11 etretinate-treated patients, but only 4 of 14 placebo-treated patients, remained in remission. Our patients had a much shorter period of time for induction of remission and were followed in remission for only half as long. Thus, our results and those of Lassus *et al.* (1983) show that the use of a high dose of etretinate to induce remission and subsequent maintenance on a lower dose is effective in the management of palmo-plantar pustular psoriasis.

Although there was some 'carry-over' of side-effects from the high-dose part of the trial, patients tolerated the low-dose etretinate well and our failure to find any serum abnormalities suggests that such a regimen is safe.

## ACKNOWLEDGMENTS

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***Society Proceedings***

# Proceedings of the 236th Meeting of the Netherlands Society for Dermatology and Venereology, Amsterdam, 1 February 1986

PRESIDENT: P.A.J.M.M. DE COCK
HOST: PROFESSOR DR R.H.CORMANE

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## SUMMARIES OF PAPERS

### Comparison of the *in vitro* dissolution rates of five oral preparations of 8-methoxypsoralen

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We compared *in vitro* dissolution rates of five different 8-methoxypsoralen (8-MOP) preparations: (i) Meladinine tablets (Norway); (ii) Oxsoralen capsules with 8-MOP dissolved in liquid (Austria); (iii) Meladinine tablets (Germany); (iv) Oxsoralen capsules (U.S.A.); and (v) Geroxalen capsules (Austria). Only preparations iii, iv and v are currently available in The Netherlands. Dissolution rates were determined with the rotating basket method, described in the *United States Pharmacopeia* (*Edn. 21*) (*USP*), and a new column type method. With both methods we obtained similar results.

T25, the fraction of the total amount of 8-MOP dissolved after 25 min, was as follows: i, T25 = 96%; ii, T25 = 92%; iii, T25 = 61%; iv, T25 = 44%; v, T25 = 6%. These results are in good agreement with biopharmaceutical data known from *in vivo* studies.

The *USP 21* requires no determination of the dissolution rate for 8-MOP tablets. In view of *in vivo* data, we postulate that an 8-MOP tablet or capsule should meet a requirement of T25 = 80%.

None of the three 8-MOP preparations available in The Netherlands meets this requirement.

### The effect of ketanserin on Raynaud's phenomenon in scleroderma patients. A double-blind placebo-controlled study

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A diverse body of evidence suggests that 5-hydroxytryptamine (5-HT) is strongly implicated in the aetiology of peripheral vasoconstrictive disease, including Raynaud's phenomenon (RP).

The effect of ketanserin, a specific 5-HT antagonist, was investigated in a placebo-controlled, double-blind study in 17 patients with progressive systemic sclerosis, CR(E)ST-syndrome, acrosclerosis and mixed connective tissue disease associated with RP. Quasi-continuous digital arterial rest flow was measured by an ECG-triggered plethysmograph, while the fingers were warmed from baseline to 38°C and cooled with $CO_2$ gas until symptoms of RP appeared. These assessments were made before treatment and again after 6, 12 and 24 weeks of treatment with either placebo or ketanserin 40 mg three times daily.

Plethysmographic measurements demonstrated that keranserin stimulates recovery from the induced effect of cold and of the digital flow. This could mean that 5-HT modulates resting skin flow and cold-induced vasoconstriction and that 5-HT antagonism by ketanserin has a beneficial effect on the intensity and duration of RP in scleroderma patients.

## Treatment of leg ulcers by skin transplantation

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The effect of transplantation therapy on leg ulcers was evaluated retrospectively in patients treated in our in-patient department from 1974 to 1984.

During that time, 630 patients were treated for leg ulcers, most of them being of arterial and/or venous origin. In the period 1974–1975, almost all were treated conservatively (without skin transplantation). The healing time of the ulcers was 10·1 weeks (s.d ± 8·0). From 1976–1980, patients were treated with a transplantation: half by a split skin thickness transplantation, and half by an autologous punch-biopsy transplantation. The healing time of the ulcers decreased to 7·5 weeks (s.d. ± 4·8). In 1980, a special pre-transplantation regimen was added and modified in 1982. Wet dressings with physiologic saline solution, acetic acid 1–2% solution and iodide solution were applied 3 times daily each for 2 days and resulted in a clean, well-granulating wound bed after 6 days Transplant therapy had a good result, the healing time of the ulcers decreasing to 4·3 weeks (s.d. ± 1 6).

## Skin immune system (SIS)

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Kondo (1922) was the first to describe intra-epidermal lymphocytes. Fichtelius, Groth & Liden (1970) suggested the skin to be a 'first level lymphoid organ' in which non-competent lymphoid cells become competent during or shortly after a stay in the epidermis. Streilein (1978) coined the term 'skin associated lymphoid tissues' (SALT) to describe Langerhans cells (LC), epidermotropic recirculating T lymphocyte subpopulations (homing T-lymphocytes), keratinocytes producing epidermal thymocyte activating factor, and the skin draining peripheral lymph nodes, thereby including vascular high-endothelial cells with the capacity to capture lymphocytes passing through the blood.

LCs form part of a widely distributed family of dendritic accessory antigen presenting cells and the organ specificity of this subset for stratified squamous epithelial tissues does not necessarily imply a distinct and tissue-specific type of immune function. High endothelial venules (HEV) that are supposed to be necessary for organ-specific T-cell migration directing mechanisms have not been described in healthy skin. Definite proof for intra-epidermal T cell differentiation *in vivo* is still lacking. Finally, skin-draining lymph nodes do not exclusively receive skin lymph.

We propose redefining the immune response-related cells in the skin as the skin immune system (SIS). In addition to the cells included in Streilein's original definition of SALT, one may also add a number of other immune response-related cells that normally populate the (epi)dermal tissues. SIS allows description of the immune system operating in normal skin according to its cellular components. There is no claim for organ-specificity of such an immune network. The cells also to be included are type I and II mast cells, tissue macrophages, granulocytes, indeterminate cells, veiled cells, vascular endothelial cells and afferent lymphatic endothelium starting in the dermis. All these cells taken together with those comprising SALT, but excluding lymph node tissue, form an intricate and complex system that we propose to call the 'skin immune system' (SIS).

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## Dermatoscopy

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Dermatoscopy allows appreciation of fine detail not normally seen with the naked eye. To study 'normal' skin, standard magnifications of × 6 to × 40 were taken from the various body regions in which the skin shows characteristic differences. Six age groups were compared (1–100 years), and sex differences were also registered. Hair growth patterns and mucosal surfaces were also subjected to dermatoscopy. Typical signs of several types of skin disease were photographed with special attention to the fine detail of primary and secondary lesions.

With the existing techniques of UV, IR and fluorescence photography, dermatoscopy can be used to develop new diagnostic possibilities. In addition, dermatoscopic photography provides the clinical instructor with a new teaching aid.

## Sensory loss following cryosurgery

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One hundred and eighty skin lesions in 169 patients who had been treated by cryosurgery were tested during follow-up by means of the 'graded bristle technique'.

Mild, usually transient, sensory loss was found in a proportion of patients. This sensory loss did not appear to be dependent on differences in cryosurgical technique or on complications during wound healing, but was dependent on the body site of the skin where cryosurgery had been performed.

The most likely explanation for the differences in degree and duration of the sensory loss is the distance between axon damage and the cell body, which influences the rate of regeneration. This distance is greater on the trunk and extremities, where sensory loss is most evident.

## Skin manifestations of the acquired immune deficiency syndrome (AIDS)

H.J.Hulsebosch

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An infection with the HTLV III (LAV) virus can lead to seroconversion without disease, the lymphadenopathy syndrome (LAS), the AIDS-related complex (ARC) and AIDS.

AIDS is dominated by life-threatening opportunistic infections of internal organs, while Kaposi's sarcoma (KS) is the most important skin manifestation, being part of the Center of Disease Control (CDC) definition. The clinical diagnosis of KS can be difficult, especially in an early stage. Although no curative treatment exists, the dermatologist can offer electrocoagulation of cosmetically unacceptable lesions on the face and intralesional injections of cytostatic drugs. Besides KS, there is a growing list of dermato-venereological diseases associated with, and whose expression is influenced by, the immune deficiency state of the patients.

Recently, a review was published by Penneys and Hicks (1985). To this, the yellow nail syndrome (Chernovsky & Finley, 1985) and a papular eruption (James *et al.*, 1985) need to be added.

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## Relationship between skin diseases and behaviour variables

W.W.VAN DER SCHAAR

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Emotional factors are important elements in the development and prolongation of many dermatological disorders, but there is considerable disagreement about the degree to which emotions play a role. The term 'psychosomatic dermatosis' is insufficient when referring to conditions in which emotional factors play the essential pathogenetic role.

Several attempts have been made to classify psychosomatic dermatoses according to the relative importance of emotional elements, varying from dermatoses 'exclusively' emotional in origin to dermatoses 'sometimes' influenced by emotional factors (Rook & Wilkinson, 1982). All classifications contain inconsistencies and overlaps between the categories. The same skin disease, such as psoriasis, can be perceived differently, and thus classified differently, by different authors.

To overcome this general dissatisfaction with the current diagnostic classification scheme of psychosomatic skin diseases, the author suggests another classification based on the behaviour of the patient as a consequence of that particular dermatosis rather than the dermatological diagnosis *per se*. The patient's behaviour then may be influenced by seven behaviour patterns which consist of: (i) pain/itching; (ii) habit formation; (iii) disfigurement; (iv) delusions/psychosis; (v) fixation to the skin; (vi) non-compliance, and (vii) emotions: verbal/non-verbal. Consequently, it is the patient who determines whether his skin disease can be approached by the psychosomatic method, instead of the dermatologist or psychotherapist. This view is in line with the heuristic psychosomatic emphasis on approach, involving the patient's behaviour in coping with his skin disorder, rather than nosological categories with a so-called psychogenetic cause.

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## Treatment of vitiligo with L-phenylalanine and light

R.H.CORMANE, A.H.SIDDIQUI, W.WESTERHOF, R.H.B.SCHUTGENS*, R.HU AND V.I.MOHAN

*Departments of Dermatology and *Paediatrics, Academic Medical Centre, Amsterdam*

It has been reported that normal melanin synthesis occurs in phenylketonuria (PKU) skin with the administration of tyrosine and also with the incubation of UV-light irradiated PKU skin. This therapy was tried in vitiligo. In a preliminary study, tyrosine in combination with UVA irradiation was tried in vitiligo for 6 months with no benefit. L-phenylalanine (Phe), was then tried at a dose of 50 mg/kg body weight followed by UVA/sunlight exposure. Since the peak serum level of Phe occurred 30–45 min after its ingestion, UVA/sunlight is given at this time.

Six months after this therapy, repigmentation had occurred in some of the vitiliginous macules. An increased dose of 70 mg improved the results and the dose was increased further to 100 mg which gave excellent results. Perifollicular repigmentation and repigmentation from the edges of the vitiliginous macules were observed. More than 90% of the 200 patients benefited from this therapy. Optimal repigmentation of the vitiliginous macules was noted in early disease, but prolonged treatment still induced repigmentation in long standing cases. Repigmentation occurred mainly in areas rich in follicles. Phe loading in increasing doses did not influence the serum tyrosine levels.

The mechanism of repigmentation may be the stimulatory effect of Phe or one of its metabolites in combination with UVA/sunlight on melanocytes still present in the vitiliginous skin. Side-effects from oral ingestion of Phe in this dose schedule are not likely. Vitiliginous skin of longstanding cases becomes less sensitive to sunlight and normal skin tans very well during Phe-UVA therapy. Contra-indications are PKU and pregnancy.

## CASE REPORTS

## Ectrodactyly, ectodermal dysplasia and cleft lip-palate syndrome (EEC syndrome)

R.F.H.J.HULSMANS, M.J.VAN SCHOONEVELD, K.KLOP, J.H.SILLEVIS SMITT, J.W.DELLEMAN AND W.WESTERHOF

*History*. Female, aged 22 years, born with multiple congenital defects. Her mother had ectrodactyly (Fig. 1), syndactyly, clinodactyly, ectodermal dysplasia, occipital partial alopecia and cleft lip and palate.

*Examination*. (1) Osseous and soft tissue anomalies: 6 months retardation of skeletal growth at the age of 3½ years, flat nose, cleft lip and palate, soft tissue syndactyly III/IV/V on right foot, short and broad hands with low implantation of the thumbs, radiographically broad proximal phalanges and double distal phalanges of both thumbs and 1st toe of the right foot (Fig. 2).

(2) Ectodermal dysplastic anomalies: occipital partial alopecia, dacrostenosis, absent or hypoplastic Meibomian glands (as shown by absent gland orifices at slit-lamp examination) leading to corneal clouding, vascular ingrowth of cornea, photophobia and lowered visual acuity; conical-shaped teeth, partial hypo- and anodontia, mammary hypoplasia, hypohidrosis (palmar biopsy: hypoplastic sweat glands) and xerodermia.

(3) Neurological disturbances: slight mental retardation, slow irregular EEG-pattern with bilateral, paroxysmal instability at the age of 1 year, conduction-type hardness of hearing.

*Discussion*. The EEC syndrome is inherited as an autosomal dominant trait with incomplete penetrance. The variable expression of this syndrome may be assumed by the fact that ectrodactyly was present in the mother but absent in our patient (Küster, Majewski & Meinecke, 1985). Our case is remarkable in the

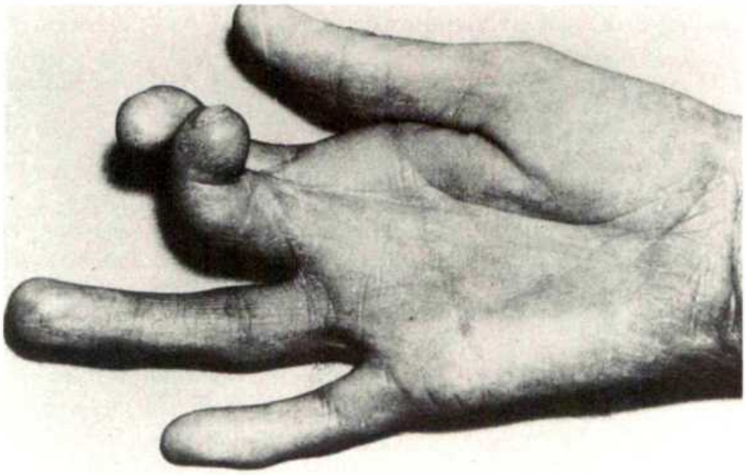

FIGURE 1. Hands of the mother of the patient showing ectrodactyly (lobster claw deformity) and clinodactyly of digit II of the right hand.

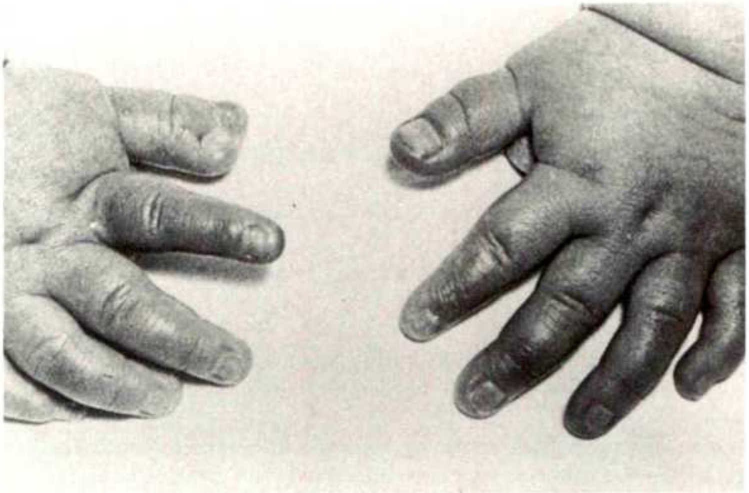

FIGURE 2. Hands of patient at the age of 2 years. Note short broad hands with low implantation of thumbs, double distal phalanges clearly visible on right thumb and absence of ectrodactyly.

absence of Meibomian gland orifices leading to severe keratopathy with visual disturbances (Mondino *et al.*, 1984).

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## Hereditary papulo-translucent acrokeratoderma as a variant of and in association with palmoplantar punctate keratosis

F.S.DE WIT AND R.F.H.J.HULSMANS

*History.* Black female, aged 44 years, suffering from palmoplantar callosities for the last 20 years. Slight palmoplantar hyperhidrosis but no other associated symptoms, normal hair, normal nails, normal dentition, normal IQ, no eye abnormalities. Her father showed an identical eruption on his feet.

*Examination.* Two types of cutaneous anomalies were present. One was localized to both palms and soles and consisted of small keratotic papules which could be removed mechanically, leaving a central crater. This picture was consistent with the clinical diagnosis of palmoplantar punctate keratosis.

The other type of lesion was also localized to the hands and feet but distributed differently. On the hands, translucent, yellowish, firm papules were found, localized to the thenar, extending to the hypothenar and on the dorsal surfaces of the interphalangeal joints. Diffuse hyperkeratotic plaques were arranged bilaterally along the foot margins extending to the achilles-tendon. This picture was consistent with the differential diagnosis of acrokerato-elastoidosis or papulo-translucent keratoderma.

*Histology.* A hyperkeratotic epidermis was present, the dermis was without abnormalities, in particular the elastic fibres were normal, thus excluding the diagnosis of acrokerato-elastoidois.

*Discussion.* Our patient showed a combination of skin diseases which are known as distinct entities, but have not yet been described together.

## Phakomatosis pigmentovascularis type IVb

W.WESTERHOF, P.FLEURY* AND J.H.SILLEVIS SMITT

**Department of Neurology*

*History.* Female, aged 11 years, born with multiple pigmented macules on the left side of the face, trunk and left arm. Soon after birth, patchy dark hairs developed on the left side of the scalp. Convulsions started at the age of 3 months. She hardly used her left arm because it seemed painful. At school she exhibited a moderate mental retardation. Family history was negative.

*Examination.* Multiple naevi spili on the left side of head, neck, trunk (Fig. 3) and left arm, were not clearly related to a dermatome. The hairs arising from the dark components of the hyperpigmented lesions of the scalp were black, in contrast to the normal blond hairs elsewhere. A naevus flammeus was seen on the right side of the back. On the upper part of the trunk a linear naevus verrucosus was noticed. A faint Mongolian spot occurred on the lower back.

She had a thoracic scoliosis (convex to the left side) and a hemiatrophy of the left side of the body. The left iris was more pigmented than the right. There was hypertrophy of dermatomes Th4-10 on the left side and motor impairment of the left facial muscles.

*Histology.* Biopsy taken from the naevus flammeus region showed no pathology. Biopsy from the naevus spilus showed lentiginous areas and nests of pigmented naevus cells.

*Investigations.* EEG showed a very slow background pattern, especially on the left side, with an epileptic focus in the left temporal lobe. Skull X-ray was normal. An X-ray of the spine confirmed the scoliosis, without abnormalities of the vertebrae and normal foramina intervertebralia. Cerebral angiography was normal. A cerebral CT scan revealed atrophy of the left hemisphere.

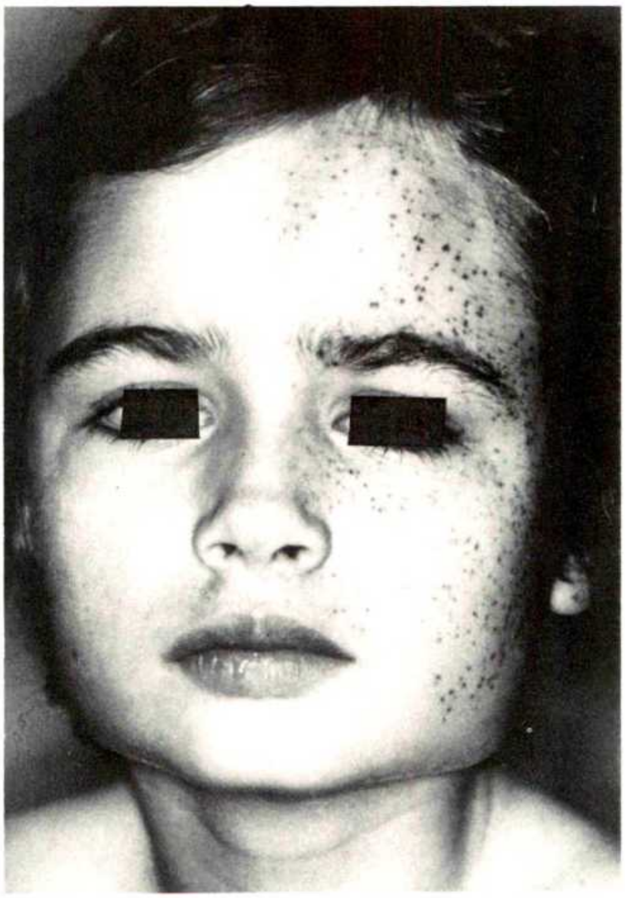

FIGURE 3. Naevus spilus on the left side of the face.

*Treatment*. The convulsions have been controlled with carbamazepine. Several therapies for the hypertrophy were unsuccessful.

*Discussion*. These pigmented and vascular lesions presenting together with systemic disturbances have not yet been reported in this way. The disorders in this patient can be grouped under the heading of phakomatosis pigmentovascularis type IVb (Hasegawa & Yasahara, 1985).

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## IgA isotypes in linear juvenile IgA dermatosis

J.H.SILLEVIS SMITT AND E.J.M.BAKKERS

*History*. A 2-year-old boy presented with a disseminated, non-pruritic, skin eruption. The lesions started in the anogenital region but soon involved almost the entire skin surface. No systemic symptoms

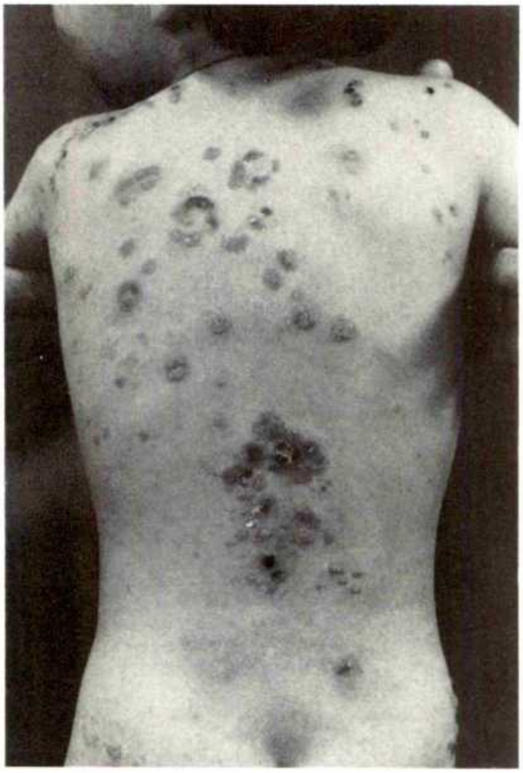

FIGURE 4. Annular-arranged blisters with clear fluid on the back.

were present, especially no gastro-intestinal problems. Oral antibiotics did not result in improvement of the skin condition.

*Examination.* An eruption was seen consisting of bullae filled with clear fluid on an erythematous base, dispersed over the whole skin surface, which was most severe on the trunk, the anogenital region and the proximal parts of the extremities. Some bullae were annular or surrounded by a crusted area (Fig. 4). Nikolsky sign was negative; no mucous membrane lesions were found.

*Histology.* Sub-epidermal oedema and blister formation with an infiltrate consisting of neutrophils were most pronounced in the tips of the papillae. Slight exocytosis was present.

*Immunofluorescence.* Direct: linear deposition of IgA was seen along the basement membrane. No staining was observed with antibodies to the secretory component of IgA. Indirect: negative.

*Discussion.* The IgA deposits in linear IgA disease are different from those in dermatitis herpetiformis since they contain no secretory component (Leonard *et al.*, 1984). Absence of secretory IgA could be demonstrated in this case of linear juvenile IgA dermatosis.

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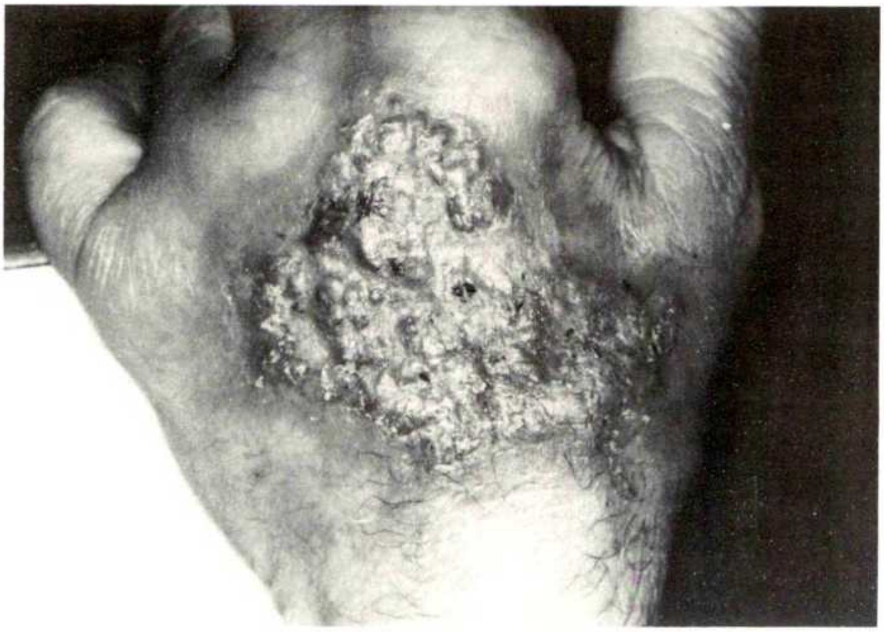

FIGURE 5. Clinical picture of the lesion on the left hand.

## Actinomycosis

I.H.Go AND H.Neering

*History*. Male, aged 81 years. In the autumn of 1984, he developed a slowly growing lesion on the back of the left hand, following an accident with a saw.

*Examination*. On the back of the left hand a sharply demarcated eruption was seen, composed of erythematous papules, nodules and some pustules. There was a purulent discharge, in which yellow grains, 'sulphur granules', could be seen.

Around this eruption there was an erythematous border of about 2 cm. (Fig. 5) There were no palpable lymph nodes in the axillary fold.

*Histology*. Cavities filled with pus and surrounded by oedematous granulation tissue were present in the dermis. In one of these cavities a colony was found in which the organisms were arranged in palissade formation and from which hyphae radiated. This is the typical picture of actinomycosis.

*Discussion*. Actinomycetes are bacteria that produce filamentous branched hyphae and can be secreted by the infected wound as yellow grains, the so called 'sulphur granules'. Pathogenic actinomycetes are found in the soil as saprophytes. On historical grounds actinomycosis is usually dealt with under the heading of mycotic infections.

One of several antibiotics can be used for treatment. Erythromycin 2 g/day was given for 5 months until healing was complete.

## Cat scratch disease

M.M.D.van der Linden

*History.* Female, aged 42 years, who had a painless swelling in the left groin for 14 days; during the last 7 days, painful red swellings on the lower legs and a spreading itching rash over the whole body had developed. The patient complained also of general malaise and a fever of 39°C. No medication had been taken. She kept two cats and had not recently visited a tropical area.

*Examination.* A tense swelling of 5 cm diameter was palpable in the left groin. On the right upper leg a cat scratch was noted. Partially confluent erythematopapulous lesions were present all over the body, including face, palms and soles. On the lower legs we saw several erythematous nodules.

*Differential diagnosis.* I. Lymphogranuloma venereum, syphilis second stage, lymphoreticulosis benigna (cat scratch disease), lymphoreticulosis maligna. II. Erythema nodosum.

*Histology (of an erythematopapulous lesion).* In the upper and lower part of the dermis there was a mainly perivascular infiltrate consisting of neutrophils, lymphocytes and plasma cells.

*Investigations.* ESR 44 mm/h, no other abnormalities. Hepatitis B antigen: negative. Blood tests for syphilis were negative. STD screening: negative. Dark field examination of a papulous lesion: negative. LGV antibodies: no titre increase. PPD: negative. An intradermal test with cat scratch antigen showed, after 4 days, a papulopustule with a diameter of 5 mm, the border of which was erythematous.

*Course.* After 20 days the swelling in the groin had disappeared. The erythema nodosum and the erythematomaculopapulous lesions also healed spontaneously. The patient had not received any specific therapy.

*Discussion.* The causative agent of cat scratch disease has not been identified. It is most probably a microorganism which penetrates the skin after a cat scratch or bite. The intradermal test with cat scratch lymph node antigen (Foshay- or Hanger-Rose test: almost 100% specificity) provided us with the diagnosis. The erythema nodosum as well as the maculopapular lesions can be cutaneous manifestations of the disease. The disease requires no special therapy.

## Erosive adenomatosis of the nipple

G.R.R.Kuiters, R.F.H.J.Hulsmans and P.L.M.Lichtveld

*History.* Female, aged 19 years, had a 5-month history of an initially serous and later bloodstained discharge from the right nipple. The nipple itched and felt indurated. She used oral contraceptives.

*Examination.* A papilliferous erosive nipple with slight induration of the right areola was seen.

*Histology*. Proliferation of sub-epidermal lactiferous duct-like structures with luminal columnar cells surrounded by flat myo-epithelial cells was found (Fig. 6).

*Treatment*. The lesion was excised. The result was cosmetically and functionally satisfactory.

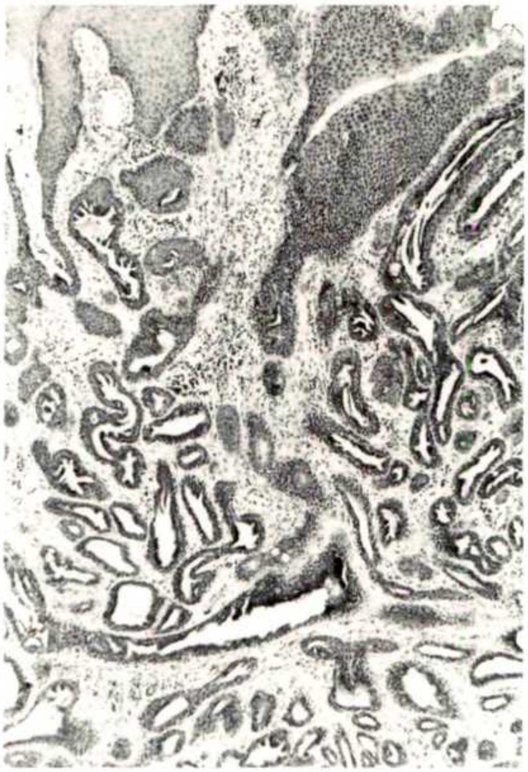

FIGURE 6. H & E staining (original × 16) shows multiple sub-epidermal lactiferous duct-like structures.

*British Journal of Dermatology* (1986) **115**, 595–599.

***Case Reports***

# Bazex paraneoplastic acrokeratosis: a case report and response to Tigason

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## SUMMARY

A clinically typical case of Bazex acrokeratosis neoplastica is presented. This case is unusual in that the syndrome developed two years after a neoplasm was diagnosed. Almost complete clinical clearance was obtained with oral Tigason®, making it the first recorded case to improve without removal of the underlying neoplasm.

Acrokeratosis paraneoplastica was first described by Bazex *et al.* (1965). Its typical features and development have been well reviewed by Bazex and Griffiths (1980) and Pecora *et al.* (1983). Erythematous scaly involvement of the extremities, ears, and the bridge of the nose is associated with a malignancy, most frequently in the laryngo-pharyngeal region. It is usually, but not exclusively, seen in Caucasian males of 40 years of age and over and there is only rare association with other cutaneous signs of malignancy—ichthyosis (Moulin, Valignot & Boucher 1975; Dupre *et al.* 1976) and bullous pemphigoid (Rosner, Nurse & Dowling, 1984). It has been found associated with squamous cell carcinoma involving lymph nodes of the neck without an identifiable primary site in 10 of 50 cases (Pecora *et al.*, 1983). The condition may clear with removal of the primary neoplasm but recurs with regrowth of the neoplasm or metastasis to the neck, but not distant metastases. Bazex (1979), quoting a thesis by Bilbeissi, reported a patient with a low serum vitamin A level, while Hagedorn and Stengel (1983) reported a patient with a vitamin A defect not corrected by oral or intramuscular vitamin A. Resistance of the condition to any treatment (apart from removal of the underlying neoplasm) is typical.

## CASE REPORT

The patient, a 64-year-old process worker employed in an ink factory, presented in August 1984 with a history of a dry itchy rash affecting his hands from June 1983. Later, his feet, nose and ears became involved. At about the time the hand involvement appeared, the patient had accidentally spilt a chemical solution 'Parmentol A 23' inside his gloves. Local steroids had not helped his condition.

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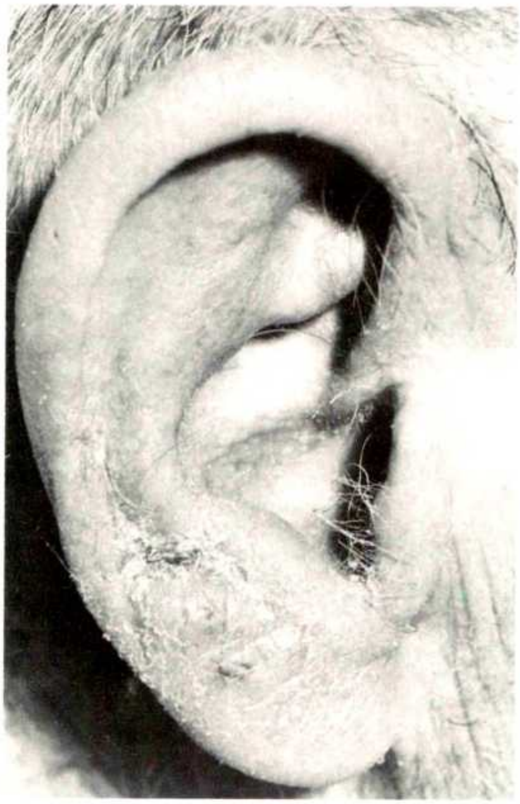

FIGURE 1. Patient's ear showing erythema and scaling.

On examination, he was a thin man who was centrally cyanosed with erythema and scaling of the nose and ears (Fig. 1). His palms showed a keratoderma with a pinpoint, honeycomb-like pattern and central clearing (Fig. 2). There was clubbing of the finger nails with dystrophy and partial loss of the finger nails of the 4th fingers, while the toenails were yellowed and thickened. In addition, he had intense scaling and erythema of both surfaces of the hands and feet (Fig. 3) and of his knees. A lymph node was palpable in his left supra-clavicular area. Clinically, this suggested Bazex paraneoplastic acrodermatosis. Review of his previous history revealed the excision of a lymph node from the left side of his neck in August 1981. The node contained squamous cell carcinoma. He had smoked 30 cigarettes a day for 40 years and had a morning cough. There was some shortness of breath on heavy exertion at work. Chest X-ray suggested old apical tuberculous lesions with basal shadowing suggesting interstitial fibrosis.

Normal or negative findings included sinus X-ray, barium meal, bronchoscopy, ear, nose and throat examination under anaesthetic and sputum culture, including TB. Lung biopsy showed alveolar wall cells mildly thickened by connnective tissue, and alveolar lining cells were prominent. These investigations thus revealed a secondary squamous cell carcinomatous deposit in the neck with an unknown primary, and interstitial lung disease. At this stage, and subsequently, he has refused further invasive investigations.

In 1984, sputum cytology was normal and chest X-ray unchanged. Skin biopsy from the hand showed psoriasiform hyperplasia with a few foci of spongiosis. Adjacent to the spongiotic foci

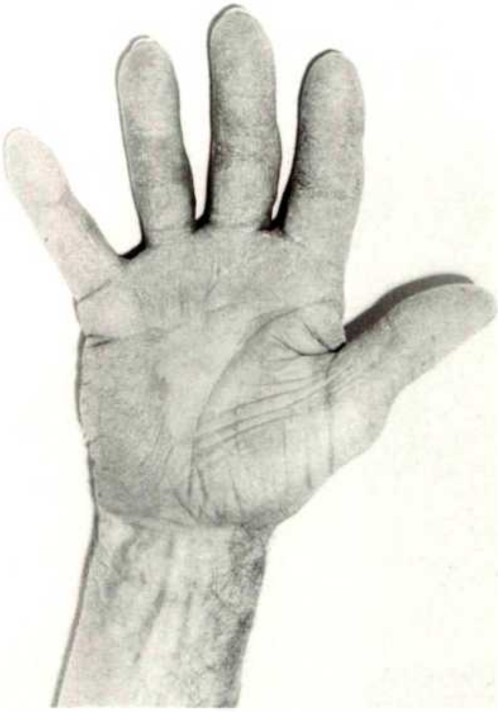

FIGURE 2. Patient's hand showing keratoderma.

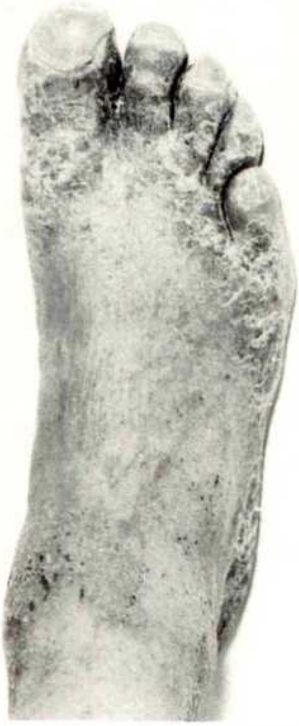

FIGURE 3. Intense scaling and erythema of dorsum of foot.

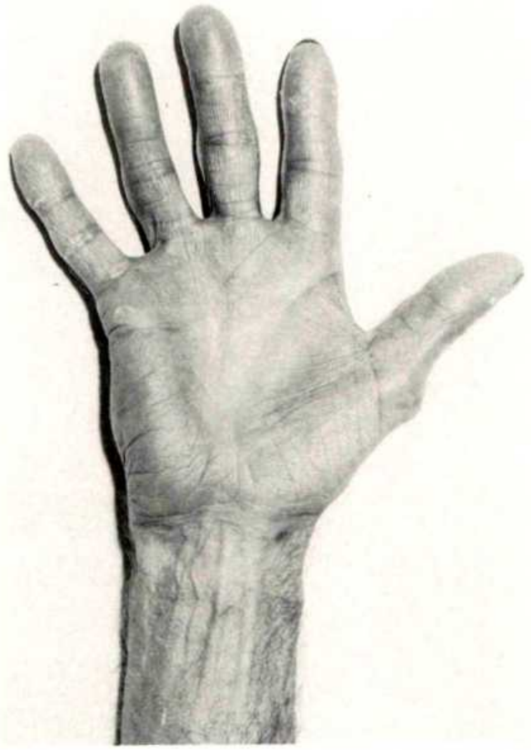

FIGURE 4. Patient's hand after 2 months of oral Tigason® showing almost complete clearing.

were limited interface changes with loss of basal cells. The upper dermal inflammatory infiltrate comprised lymphocytes, histiocytes and a few eosinophils. Thus, he had spongiotic and focal lichenoid changes.

By August 1985, the itching had become very distressing and Tigason® (etretinate) 35 mg daily was started. After 2 months, the skin eruption was much improved (Fig. 4). His appetite and weight (56 kg) were maintained.

## DISCUSSION

Clinically, this patient presented with a typical case of Bazex acrokeratosis paraneoplastica, the development of which was also typical as it was associated with metastatic squamous cell carcinoma of the neck without an identifiable primary. Atypical, however, was its development 2 years after the neoplasm was first detected. At present, the patient seems otherwise well apart from his interstitial lung disease and does not have an identifiable carcinoma, as is usually present at the advanced stage of this syndrome. Because of the ichthyotic aspect of the syndrome he was treated with Tigason and is the first reported case to improve without the removal of an underlying neoplasm. Retinoids are known to have anti-neoplastic activity which may have been helpful in this case (Bollag, 1985). Tigason is also considerably more potent than the vitamin A used by Hagedorn and Stengel (1983).

## ACKNOWLEDGMENTS

My thanks are due to Dr G.H.Mason for interpreting the histology and to the staff of the Auckland Hospital Photographic Section.

## NOTE

Since this report was accepted for publication, the patient was admitted to hospital in July 1986 with a relapse of his skin condition, cachexia, bed sores and jaundice. He died 3 weeks later. He had stopped Tigason treatment early in 1986. At autopsy, there was disseminated metastatic squamous cell carcinoma involving the liver, ribs and lumbo-sacral spine, but no evidence of a primary, particularly not in the upper laryngo-tracheal area.

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# Acute HTLV III infection associated with exanthema, diagnosed by seroconversion

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## SUMMARY

We report three homosexual men who developed malaise, fever and a roseola-like exanthema which disappeared over 2 weeks. During 3 months follow-up, all three patients felt well and had no symptoms. HTLV III seroconversion was detected several weeks after the acute HTLV III infection. In two of the three cases the Western blot technique was able to detect HTLV III antibodies before they were detectable by the ELISA technique.

Acquired immune deficiency syndrome (AIDS) is caused by a lymphocytopathic retrovirus known as lymphadenopathy-associated virus (LAV) (Barré-Sinoussi *et al.*, 1983), AIDS-associated retrovirus (ARV) (Levy *et al.*, 1984) and human T-lymphotropic virus type III (Gallo *et al.*, 1984). The viral infection causes a broad clinical spectrum from a carrier state with antibodies against HTLV III to full-blown AIDS. Serological tests have made it possible to diagnose viral infection in symptom-free individuals. Actual seroconversion has only been reported sporadically (Cooper *et al.*, 1985; Lindskov *et al.*, 1986) and present knowledge of acute HTLV III infection is rather vague. We here report three cases of acute HTLV III infection who were followed closely during seroconversion.

## CASE REPORTS

### *Case 1*

A 23-year-old male homosexual was referred because of 2 weeks of generalized symptoms including fatigue, fever (38–39°C), a sore throat, abdominal pain and transient diarrhoea. Two days before admission he had developed a non-itchy, non-scaling rash on the upper trunk and face. After development of the rash the patient was treated with sulphamethoxazole-trimethoprim 400:800 mg b.i.d. by his general practitioner.

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*Clinical examination.* A dispersed roseola-like exanthema was present on the patient's face, neck, shoulders and upper trunk. A few lesions were noted on his arms and the palms of his hands. An individual macula was 3–6 mm in diameter with a well defined border and central erythema. Lesions were confluent on the breast. A diffuse enanthema was present in the oral cavity. There was microadenitis in the groins and axillae.

*Exposure.* The patient had had frequent sexual relations with different partners up to the onset of the disease, but no clear-cut information was available for estimating the time of virus exposure.

*Laboratory investigations.* There was a thrombocytopenia of $49 \times 10^9$/l (normal 150–$350 \times 10^9$/l). The total leukocyte count was $3{\cdot}3 \times 10^9$/l (normal $3{\cdot}0$–$9{\cdot}0$ $10^9$/l) with a lymphocyte count of 8% (normal 15–50%).

The Wassermann reaction was negative. The total CMV antibody titre was 1:300, whereas the IgM antibody titre was 0. Serum aspartate-aminotransferase was elevated at 59 U/l (normal 10–40 U/l). The T helper/T suppressor ratio was normal (1·6).

Tests for antibodies to HTLV III (Lindhardt *et al.*, 1985) were negative in both ELISA and Western blot 3 and 4 weeks after onset, but 5 weeks after the onset of general malaise seroconversion was detected in both ELISA and Western blot (Table 1).

*Clinical course.* The patient recovered gradually over the next 2 weeks and has remained well during the subsequent 3 months (Fig. 1).

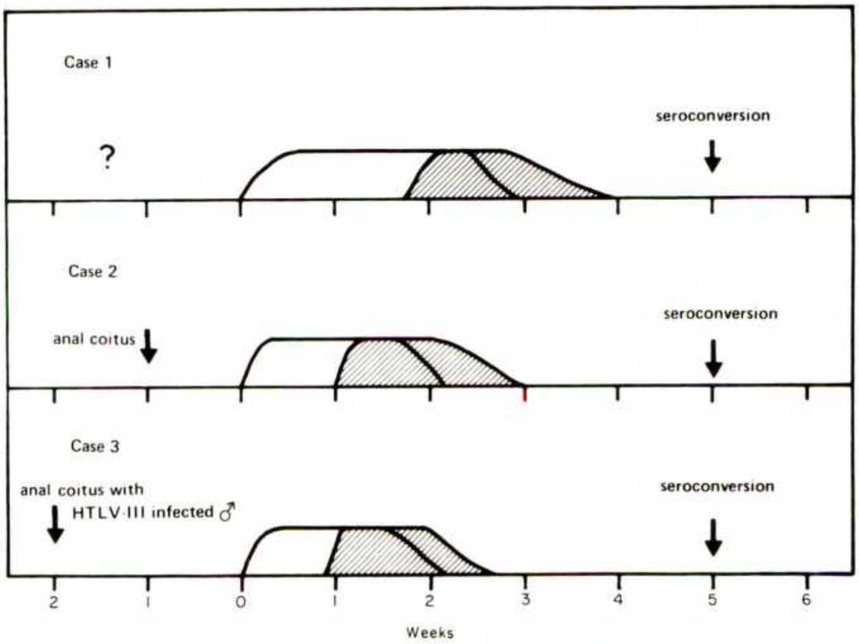

FIGURE 1. Clinical course and seroconversion in three cases of acute HTLV III infection. □ Generalized symptoms; ▨ roseola.

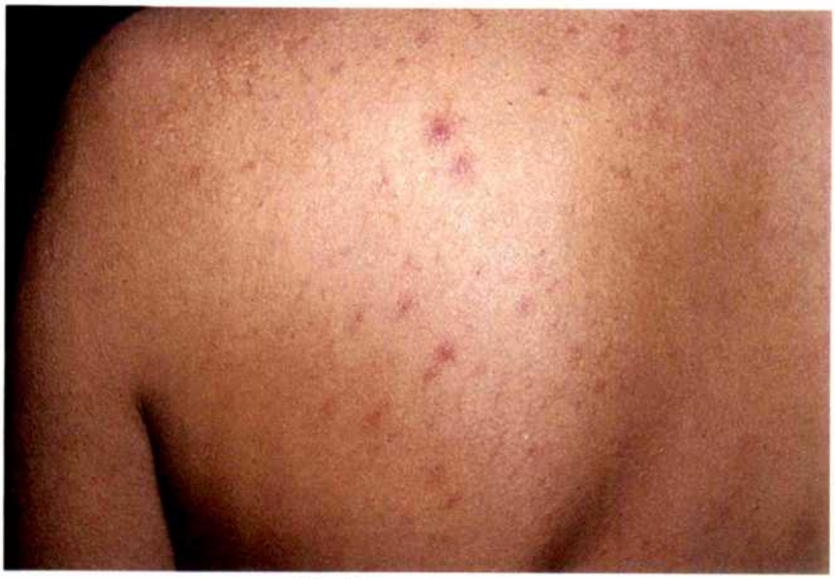

FIGURE 2. Macular exanthema on the upper trunk.

*Case 2*

A 29-year-old male homosexual was seen because of 10 days of general malaise followed by 3 days with a skin rash. The symptoms included a sore mouth and throat, poor appetite and abdominal pain. His temperature varied between 38–39°C. One week before the onset of the disease he had had anal intercourse, as the passive partner, with an American homosexual.

*Clinical examination.* A macular exanthema was present on the face, neck and upper trunk (Fig. 2). No lesions were seen on the palms or soles. Small, non-tender lymph nodes could be palpated in the axillae and groins. There was angular stomatitis and diffuse enanthema of the mouth.

*Laboratory investigations.* Thrombocytopenia $85 \times 10^9$/l was found on admission. Eleven days later the thrombocyte count was $320 \times 10^9$/l. The following tests were all normal: leukocyte count, mononucleosis infection (EMNI) test, CMV, IgM, Wassermann reaction, TPI and FTA-ABS tests.

The T-helper/suppressor ratio was 1·0, but decreased at the time of seroconversion to 0·3, followed by normal values 4 weeks later.

HTLV III antibodies (ELISA) were found to be negative and remained so until 5 weeks after the onset of the generalized symptoms. However, the Western blot showed antibody reactivity against the major core protein p24 during week 4 (Table 1).

*Clinical course.* The generalized symptoms lasted for 2 weeks and the roseola-like exanthema was apparent for a further week (Fig. 1).

*Case 3*

A 30-year-old male homosexual was referred because of an extensive macular rash of 24 h

TABLE 1. Serological investigation of three cases of acute HTLV III infection

| | Weeks after onset of symptoms | Positive/negative* | ELISA titre† | Western blot‡ bands recognized§ |
|---|---|---|---|---|
| Case 1 | 3 | < 1 | ≤ 100 | — |
| | 4 | < 1 | ≤ 100 | — |
| | 5 | 7·2 | 1500 | p24 |
| | 6 | 10·6 | 3000 | p65/p55 gp41 |
| Case 2 | −4 | < 1 | ≤ 100 | — |
| | +3 | 1·8 | ≤ 100 | — |
| | +4 | 2·3 | ≤ 100 | p24 |
| | +5 | 3·2 | 400 | p24 |
| | +6 | 3·2 | 400 | p24 |
| | +7 | 4·6 | 800 | p24 |
| Case 3 | −10 | < 1 | ≤ 100 | — |
| | +4 | 2·1 | ≤ 100 | p24 |
| | +5 | 3·3 | 400 | p24 |
| | +6 | 3·7 | 800 | p24 |
| | +8 | 8·2 | 1600 | p24 p18 |
| | +11 | 9·8 | 3200 | p24 p18 |
| | +15 | 15·6 | 12 800 | p65/p55 p24 p18 gp41 |

* Positive/negative ratios exceeding 3·0 considered positive.

† Endpoint titration, i.e. highest dilution giving optical density reading equal to or exceeding the optical density of a negative control standard serum.

‡ Sera not titrated, serum diluted 1:500.

§ p24, p18: *gag*-related proteins; gp41: *env*-related transmembrane glycoproteins; p65/p55: *pol*-related reverse transcriptase complex.

duration and a febrile illness for about one week. He complained of a sore throat and general malaise. Exactly 2 weeks before the onset of symptoms he had had anal intercourse, as the passive partner, with an HTLV III antibody-positive person. The patient denied any other sexual contact for at least 18 months.

*Clinical examination.* A widespread macular exanthema was present on his face, neck and upper trunk. Slightly enlarged lymph nodes were palpated in the axillae and groins. Diffuse enanthema was present in his mouth.

*Laboratory investigations.* Leukocytopenia of $2{\cdot}8 \times 10^{9}/\mathrm{l}$ was present when the symptoms were most severe. One week later both leukocytes and thrombocytes were normal.

HTLV III antibodies were negative at the time of admission and one week later when the patient left hospital. The test was repeated after 4 weeks. ELISA was negative, but p24 antibody was detected on Western blot (Table 1). HTLV III was cultured from the peripheral blood during the acute stage of the disease and HTLV III antigen was detected in the serum by an ELISA antibody assay (Ulrich *et al.*, unpublished observations).

On admission, rectal gonorrhoea was diagnosed by culture and the patient treated with sulphamethoxazole-trimethoprim (400:800 mg) five tablets twice daily for 2 days. This treatment failed and was therefore followed by spiramycin injection 2 g i.m.

*Clinical course.* The patient recovered in 2 weeks and the roseola disappeared during the following week (Fig. 1).

## DISCUSSION

Acute HTLV III exanthema has recently been described (Cooper *et al.*, 1985) and led us to diagnose this clinical entity specifically related to HTLV III infection. So far, there have been many reports dealing with non-specific skin manifestations associated with HTLV III infection. These include seborrhoeic dermatitis, candidiasis, fungal infections and herpes simplex infections in clinically healthy individuals and, more rarely, histoplasmosis and erythema elevatum diutinum (Penneys & Hicks, 1985; Farthing, Staughton & Rowland Payne, 1985; Mathes & Douglass, 1985; da Cunha Bang *et al.*, 1986). Kaposi's sarcoma may be seen at all stages of HTLV III infection, ranging from a discrete plaque in an otherwise healthy individual to tumours covering the trunk, the extremities and the mucosae.

In the present study, we saw three patients who developed malaise, fever and a roseola-like exanthema which disappeared over 2 weeks; during a 16-month follow-up period all three patients felt well and had no symptoms. Acute HTLV III infection may cause flu-like symptoms, with exanthema which has not been diagnosed previously. This clinical picture may be overlooked not only by the patient himself but also by the doctors who at present do not look for it routinely. It is possible that HTLV III infection most commonly results in malaise and the other symptoms described, together with an exanthema that may be very faint.

The serology in these three cases supports an HTLV III-associated acute exanthema. However, seroconversion was detected only several weeks after the onset of symptoms. It is of importance to note that in two of three cases, the Western blot technique was able to detect antibodies prior to their detection by the ELISA technique. A more valid test for acute HTLV III infection might be antigen detection during the acute viraemic phase. How often this phase is associated with general or specific symptoms, however, remains to be determined.

## ACKNOWLEDGMENTS

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# Skin lesions from hypersensitivity to cold during chronic myelomonocytic leukaemia

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## SUMMARY

A patient with chilblain lupus and chronic myelomonocytic leukaemia (CMML) is reported. The possible mechanisms of this association which appears specific to CMML are briefly discussed.

The association of chilblain lupus and chronic myelomonocytic leukaemia (CMML) in a female patient raised the question of the frequency of associated skin lesions from hypersensitivity to cold during CMML. A review of the literature revealed five cases of CMML-perniosis syndrome (Baker, 1964; Borrie, 1964; Marks, Lim & Borrie, 1969; Kelly & Dowling, 1985), including one case initially reported as chilblain lupus (Marks, 1967). This type of association is not reported in the literature with any other type of lymphoproliferative disorder.

## CASE REPORT

A 68-year-old woman was seen in the winter of 1984. She was suffering from chilblains on the fingers. These had occurred each winter for 10 years and had recently extended to involve her face and toes, leading to hospitalization. Her history included idiopathic peripheral thrombocytopenia which had developed over the previous 6 years, and for which no aetiology had yet been determined. On examination, the patient had atrophic erythematous plaques on the backs of the hands, chilblain lesions of the pulp of the fingers and toes, and a keratotic and atrophic violaceous plaque on the tip of the nose. Clinical examination was otherwise normal.

### *Investigations*

Peripheral blood: total WBC $8{\cdot}5 \times 10^9$/l, neutrophils 37%, eosinophils 1%, basophils 0%, lymphocytes 34%, monocytes 28%. Platelets $68 \times 10^9$/l. On repeated testing, the absolute monocyte count was greater than $9 \times 10^8$/l and all monocytes were morphologically normal. Bone marrow examination showed a hypercellular picture with myeloid elements increased in

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number and a decreased number of primary granules. Monocytic precursors were increased with dysplastic changes, and cells of the megakaryocytic lineage were decreased in number.

Histological examination showed lesions mainly in the dermis with telangiectatic vessels surrounded by a moderate lymphohistiocytic infiltrate and containing numerous plasma cells. Focal changes at the dermo-epidermal junction were also noted.

Immunofluorescent studies showed a lupus band of IgM in skin lesions; but none in exposed normal skin. Serum studies showed the following results: cryoglobulins negative; cold agglutinins negative; antinuclear antibodies positive at a serum dilution of 1:400; Farr test negative; complement and its fractions normal; polyclonal hypergammaglobulinemia 24 g/l.

The patient was hospitalized twice during the winter for prenecrotic lesions of the fifth finger of the right hand accompanied by partial thrombosis of the digital arteries of the fifth finger of the right hand and the fourth finger of the left hand shown on Doppler examination. There were no other signs of the progression of the leukaemia, nor of progression of the lupus. The lesions regressed under local Lenitral (trinitrine) and heat baths.

## DISCUSSION

This patient presented a perniosis syndrome which could be related to her chilblain lupus because of the presence of antinuclear antibodies and a positive lupus band. In addition to the skin lesions, there was associated CMML with no sign of acute progression.

This case is of interest firstly because the association of a lymphoproliferative disorder with chronic discoid lupus erythematosus is infrequent in the literature as compared with the association with disseminated lupus erythematosus (Miller, 1967; Harrison & Marks, 1980; Efira, Unger & Bougaenko, 1981). Secondly, the case raises the question of the frequency of skin lesions from hypersensitivity to cold during CMML, an association which in our opinion is more than coincidental. Although skin lesions during CMML are rare in the absence of acute progression (Vail, Adler & Rothenberg, 1985), our survey of the literature revealed five cases of associated 'perniosis syndrome' (Baker, 1964; Borrie, 1964; Marks, Lim & Borrie, 1969; Kelly & Dowling, 1985), including one case initially reported as chilblain lupus (Marks, 1967).

These lesions from hypersensitivity to cold, to our knowledge, have not been described in any other type of lymphopathy and would thus seem specific to CMML. In the five cases of 'perniosis syndrome', the skin lesions appeared in elderly persons, predominantly males; the haematological process appeared stable and the skin lesions appeared before CMML was diagnosed (Kelly & Dowling, 1985). In all these cases, screening for cryoglobulins and cold agglutinins was negative, as in our case. Our patient did, however, have positive antinuclear antibodies, while other reported patients were negative. Direct immunofluorescence studies were not performed in other cases.

Is it possible to explain this predisposition to lesions from hypersensitivity to cold during CMML in the absence of any sign of acute progression? It is of interest to note that McKee and Collins (1974) have demonstrated the spontaneous tendency of myelomonocytic perniosis, but not lymphocytic leukaemias, to form 'intravascular leukocyte thrombi'. In our opinion, three mechanisms may be involved in this condition. Malignant monocytic cells, because of their rigidity, large diameter and difficulty in passing through the vascular wall may cause important modifications in vascular flow in the microcirculation (Lichtman, 1973). Secondly, with respect to a mechanism specific to CMML (Solol-Celigny *et al.*, 1984), there is a polyclonal activation of B lymphocytes characteristic of this type of lymphopathy, which is revealed by a polyclonal hypergammaglobulinemia, as in our case. This secretion of autoantibodies may thus, on the one hand, account for the association of CMML and chronic lupus erythematosus, with the presence of antinuclear antibodies as in our case, and, on the other hand, explain the cause of modifications in vascular flow. Finally, there is the action of cold which has an influence on blood viscosity as has already been demonstrated adequately (Ryan & Copeman, 1969).

This explanation may well account for the preferential association of skin lesions from hypersensitivity to cold with CMML and would thus suggest that it is advisable to carry out a full blood count in the presence of any acquired perniosis syndrome in an elderly person.

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# Atypical angiomatosis in polyarteritis nodosa

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## SUMMARY

Biopsies from an area of livedo reticularis and adjacent to a leg ulcer in a woman with polyarteritis nodosa showed florid angioendothelial proliferation simulating angiosarcoma. This angioproliferative reaction has not been described previously in polyarteritis nodosa. Its microscopic differentiation from angiosarcoma is important.

The microscopic diagnosis of cutaneous lesions demonstrating florid angiomatosis can be difficult. Atypical angiomatosis with histopathological features suggestive of angiosarcoma has been described recently in type I cryoglobulinemia (Harper, Gray & Wilson Jones, 1983). We report a young woman with polyarteritis nodosa (PAN) in whom florid atypical angiomatosis was noted on histological examination of a biopsy specimen from a diffuse livedo, as well as from the region adjacent to a leg ulcer. The initial interpretation of these specimens had led to her referral for chemotherapy of angiosarcoma. The diagnosis of PAN was made on clinical and radiographic grounds. The histological finding of florid atypical cutaneous angioendothelial proliferation in areas of livedo reticularis represents a hitherto undescribed cutaneous reaction in PAN.

## CASE REPORT

A 27-year-old Caucasian female was admitted for treatment of a presumed angiosarcoma. Five months previously she had developed a small ulcer on the posteromedial left calf, which subsequently enlarged to 6 cm diameter. The following month she had a miscarriage at 6 weeks. Three months prior to admission she underwent debridement, excision and split-thickness skin grafting of the ulcer. One month prior to admission the graft edges became necrotic, with eventual sloughing of the graft. At about this time, a diffuse livedo reticularis was noted over the trunk and extremities. A random punch biopsy from an area of livedo was performed on the dorsal right upper arm. This was reported as angiosarcoma, and on review of several previous samples from the region of the ulcer, the referring pathologists felt that these also showed

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angiosarcoma. Chest X-ray, bone scan and liver-spleen scan were normal. She was referred to our institution for excision and grafting of her ulcer and for systemic chemotherapy for what was felt to be an unusual multifocal pattern.

Her past medical history included hypertension of 2 years' duration (treated with hydrochlorothiazide and nadolol), mildly elevated serum creatinine and hepatocellular enzymes, and moderate proteinuria. She complained of vague malaise, but gave no history of any other symptoms suggestive of vasculitis or collagen-vascular disease. When first seen by us, following excision and grafting of her ulcer, she had become febrile (103·2°F) and had developed pleuritic chest pain without dyspnoea or sputum production. Chest X-ray showed bilateral pleural effusions, while a radionuclide lung scan showed a low probability of pulmonary embolism. Simultaneously, she developed sharp diffuse abdominal pain with guaiac positive diarrhoea. Positive findings on examination were the presence of bilateral pleural effusions, generalized livedo reticularis and a recent graft site. Review of previous investigations revealed a blood urea nitrogen of 26 mg/dl, creatinine 1·9 mg/dl, mild elevation of hepatocellular enzymes (SGOT 60 IU/l, SGPT 119 IU/l, LDH 335 IU/l, alkaline phosphatase 105 IU/l, bilirubin normal), 3+ proteinuria and an erythrocyte sedimentation rate of 128 mm/h. The admission blood count was unremarkable, but post-operatively a leukocytosis of 22 800/mm$^3$ had developed, with neutrophilia but no shift to the left, and without eosinophils.

These findings suggested a systemic vasculitis, probably PAN. Her presentation would certainly have been unprecedented for angiosarcoma. Review of the pathology showed a florid angioendothelial proliferation. In one focus, findings suggestive of necrotizing vasculitis were noted in a medium-sized artery. In view of doubt over the diagnosis, renal and mesenteric angiography was performed to confirm the clinical impression. This revealed fixed irregularity and beading of several of the branches of the superior mesenteric artery consistent with vasculitis, in addition to incidentally-noted crossed fused renal ectopia. Subsequent laboratory data included rheumatoid factor positive at 1:320, cryoglobulins and cryofibrinogen absent, mildly elevated CH50 (304 U/ml) and C4 (85 mg/dl) with C3 normal, circulating immune complexes by Clq binding assay minimally elevated (31 μg/ml, normal < 23) (obtained after 2 weeks of treatment), and negative or unremarkable hepatitis B surface antigen and antibody, antinuclear antibodies, serum protein electrophoresis, prothrombin time, partial thromboplastin time, thrombin time, platelet count, blood cultures and B-human chorionic gonadotrophin.

Therapy was initiated with cyclophosphamide and corticosteroids. At this time, the patient developed an encephalopathy and a peripheral 7th cranial nerve lesion. Acute renal failure due to pre-existent volume depletion, radiocontrast dye load, and probably, superimposed vasculitis developed and was managed conservatively. Subsequent problems included an episode of diffuse bowel ischaemia accompanied by hypotension and further renal insult, refractory hypertension, staphylococcal sepsis related to a central venous catheter, hepatocellular enzyme elevation (after initial return to normal) probably due to non-A, non-B hepatitis, and high-grade intrinsic superior vena cava obstruction, probably catheter-related. Despite these problems, her overall condition improved gradually, with resolution of her livedo, and she was discharged on the 99th day after admission. Cyclophosphamide was discontinued approximately 12 months later, but her course since that time has been notable for recrudescent vasculitic activity, necessitating continuation of corticosteroids and resumption of immunosuppressive therapy.

### *Microscopic observations*

Biopsy from an area of livedo reticularis on the dorsal right upper arm revealed a proliferation of

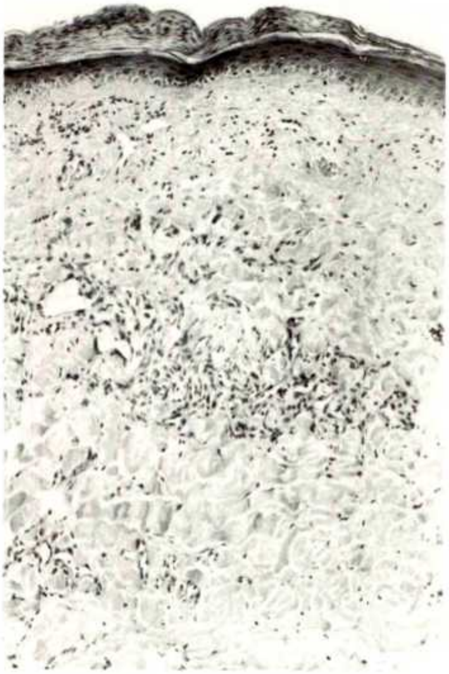

FIGURE 1. Biopsy of livedo reticularis in upper arm showing a highly atypical spindle-cell proliferation in the mid dermis (H & E; original × 125).

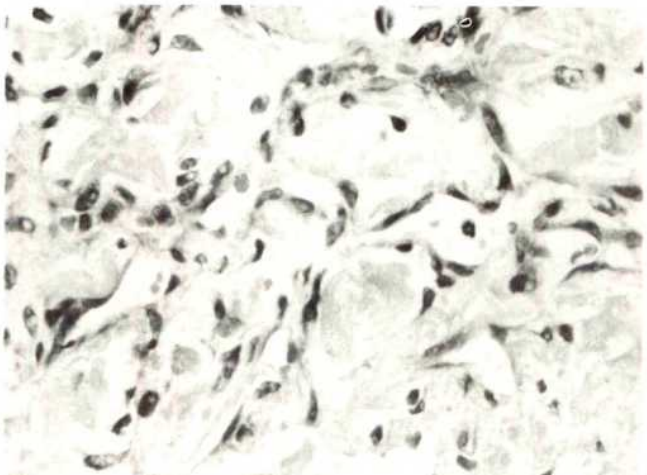

FIGURE 2. Higher power view of field in Figure 1 showing the atypical spindle cells forming anastomosing vascular spaces and scaffolding along individual collagen fibres (H & E; original × 500).

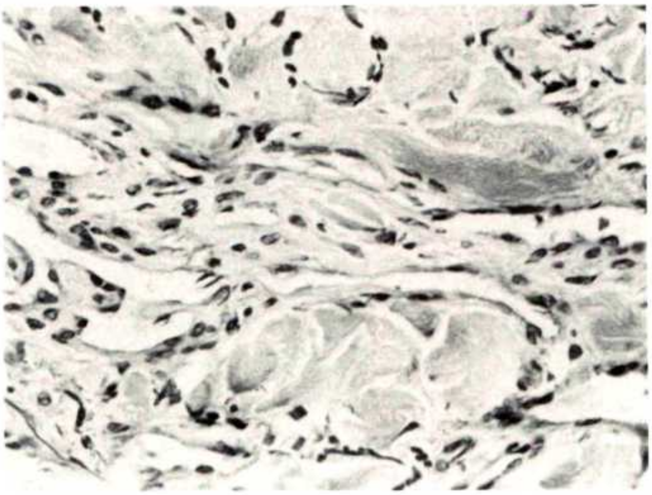

FIGURE 3. Representative photomicrograph of biopsy of leg near ulcer, revealing a similar atypical spindle-cell proliferation with anastomosing vascular spaces and scaffolding of atypical cells along individual collagen fibres (H & E; original × 500).

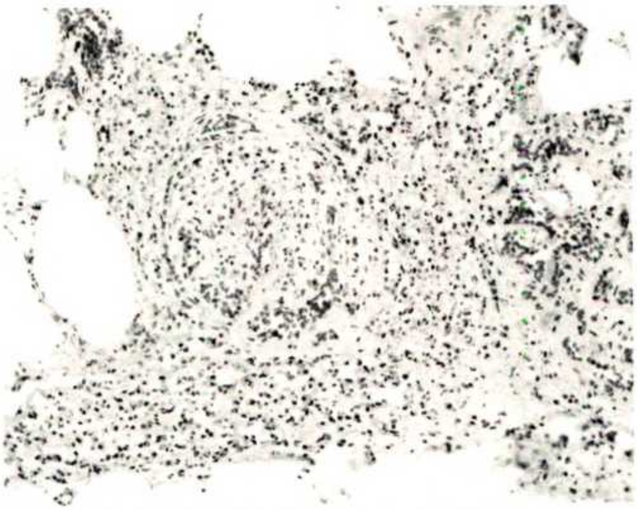

FIGURE 4. Thrombosed medium-sized artery in biopsy of leg lesion with polymorphonuclear leukocyte infiltration and focal fibrinoid necrosis of its wall (H & E; original × 200).

plump spindle-shaped cells forming irregular dilated anastomosing channels in the superficial and deep reticular dermis as well as in the subcutaneous fat (Fig. 1). The resulting network appeared to dissect between individual collagen fibres, with elongated spindle cells and endothelial-lined spaces scaffolding along dermal collagen bundles (Fig. 2). In one focus, the proliferative process surrounded a hair follicle. The overlying epidermis and papillary dermis were unremarkable. Erythrocytes were found within the small vascular channels, with occasional extravasation. Haemosiderin deposition was scanty. The nuclei of the proliferating endothelial cells were slightly enlarged, but not hyperchromatic or irregular. Mitoses were rare. An organizing thrombus was noted in a small artery in the fat. Necrotizing vasculitis was not seen. On immunoperoxidase-stained sections with antibodies directed against factor VIII (Dako Corporation, Santa Barbara, CA, U.S.A.), there was widespread staining of cells lining the small vascular channels, as well as of endothelial cells in adjacent normal vessels.

Specimens from the region adjacent to the leg ulcer revealed areas of intact epidermis subtended in many foci by a dermal and subcutaneous proliferation of spindle-shaped cells, forming lobular arrays of small vascular channels containing erythrocytes, which surrounded arterioles, capillaries and adnexal structures. At the periphery of these bundles, spindle cells dissected individual collagen fibres (Fig. 3). The lobular arrangement extended along fascial planes in the deep subcutis. The nuclei of most of the endothelial cells were plump, and the cytoplasm enlarged. There was minimal pleomorphism, however, and mitoses were rare. A sparse inflammatory cell infiltrate consisting predominantly of lymphocytes, plasma cells and histiocytes was present focally. The small and medium sized arteries present in the specimen were focally oedematous. Many of these, along with many arterioles and capillaries, contained fibrin thrombi. There were foci of intravascular papillary endothelial hypersplasia associated with these fibrin thrombi. One medium-sized artery contained a mixed acute and chronic inflammatory cell infiltrate within its wall, focal fibrinoid necrosis of the vascular wall, and thrombosis (Fig. 4). In sections containing epidermal ulceration, brisk and actively proliferating granulation tissue was the principal finding in the dermis.

In neither site was there evidence that the architecturally atypical angioendothelial proliferation was an intravascular process.

## DISCUSSION

This case is remarkable for the development of florid angiomatosis with architectural features strongly suggestive of angiosarcoma in a patient with PAN. The concomitant appearance of atypical angioendothelial proliferation in biopsies from an area of livedo reticularis on the upper arm and from the region of the leg ulcer suggests a diffuse process. Livedo reticularis is a finding which can result from numerous structural and functional causes of obstruction to cutaneous blood flow. Its histopathology is non-specific, consisting of vascular dilatation and, in many cases, variable degrees of intimal proliferation and endarteritis obliterans. Specific additional findings, if any, relate to the aetiology of the livedo. The angioendothelial proliferation seen in our patient is not a characteristic feature of livedo reticularis *per se*, and cannot be ascribed to it.

Rosai *et al.* (1976) have reviewed the histopathology of cutaneous angiosarcoma. They noted the occurrence of three distinguishable, although overlapping, architectural patterns: angiomatous, spindle-cell and undifferentiated. The architectural features noted in our patient's biopsies strongly resemble the findings in angiomatous areas of cutaneous angiosarcoma, and it was with this diagnosis that she was referred. Suggestive of this diagnosis are the presence in the reticular dermis of a freely-anastomosing network of thin-walled vascular channels lined by spindle-shaped endothelial cells, the dissection of individual collagen fibres and the tendency of the angioendothelial process to surround cutaneous adnexae without invasion. Also consistent are the presence of a grenz zone between the angioendothelial process and the epidermis, the presence of extravasated erythrocytes and the scanty hemosiderin deposition.

While these architectural similarities are striking, other features of the histopathology argue against the diagnosis of cutaneous angiosarcoma. These include the relative cytological banality of the endothelial cells, although in angiosarcoma, cytological atypia may be minimal and mitoses rare (Wilson Jones, 1960; Girard, Johnson & Graham, 1970), and the absence of true papillary tufting and of foci of necrosis. Moreover, areas consistent with spindle-cell or undifferentiated architecture were seen neither in the punch biopsy from the arm nor on extensive review of the large excisional specimen from the leg. Generally, areas characterized by each architectural pattern are found within true cutaneous angiosarcoma. While a small biopsy from a well-differentiated cutaneous angiosarcoma might fail to demonstrate such areas due to sampling error, such error is unlikely in this instance. Most importantly, the clinical presentation of the patient was inconsistent with that of cutaneous angiosarcoma, which presents as blue to violaceous plaques or nodules on the scalp or face of elderly individuals, or in the setting of chronic lymphoedema.

Rarely, Kaposi's sarcoma (KS), especially patch-stage KS, may present a microscopic picture difficult to distinguish from that of our patient, with irregular, freely-anastomosing vascular channels lined by benign-appearing endothelial cells which dissect individual collagen fibres (Gange & Wilson Jones, 1979). In general, however, the presence of spindle-cell aggregates, cleft formation, erythrophagocytosis by spindle cells, greater haemosiderin deposition and a more marked plasma cell component in the inflammatory infiltrate differentiate KS from the angioendothelial proliferation in our patient. Clinically, the leg ulcer of our patient bore no resemblance to an ulcerated nodule of KS, nor did the area of randomly-biopsied skin from the arm resemble patch-stage KS. The microscopic findings in angiodermatitis associated with arteriovenous malformations ('pseudo-Kaposi's sarcoma') may also resemble those of our patient (Marshall, Hatfield & Hatfield, 1985). However, in the case of the former, the overall architecture is more nodular, there is greater haemosiderin deposition, and occasional thick-walled vessels may be seen.

The angioendothelial proliferation noted in our patient's biopsy specimens is quite similar to that reported in a patient with type I cryoglobulinemia (Harper *et al.*, 1983). Unlike the latter, however, our patient was not noted to have angiomatous nodules, nor were cryoproteins present. It is, of course, possible that similar mechanisms were responsible for the induction of florid angiomatosis in each case.

The occurrence of an endothelial proliferative process characterized by multicentricity and the suggestion of vaso-occlusion is clinically reminiscent of the entity known as proliferating angioendotheliomatosis, either of the malignant or of the so-called 'inflammatory' type (Scott, Silvers & Helwig, 1975; Martin *et al.*, 1980). However, the microscopic findings in our case are quite different from those of proliferating angioendotheliomatosis, which is not characterized by the formation of new anastomosing thin-walled channels, but rather, demonstrates dilated capillaries lined by proliferating clumps of endothelial cells with variable degrees of cytological atypia, such areas often being contiguous to normal capillary endothelium. Clinically, proliferating angioendotheliomatosis is manifested by plaques or nodules, and was not suggested by the appearance of our patient.

Other entities considered in the differential diagnosis, but readily ruled out on histological and clinical grounds, included angiolymphoid hyperplasia with eosinophilia (Wells & Whimster, 1969; Mehregan & Shapiro, 1971; Reed & Terezakis, 1972), intravenous atypical vascular proliferation (Rosai & Akerman, 1974), intravascular papillary endothelial hyperplasia (Salyer, 1975; Clearkin & Enzinger, 1976; Kuo, Sayers & Rosai, 1976), haemangiopericytoma, pyogenic granuloma and benign haemangiomas.

It is unlikely that the patient's preceding pregnancy was associated with the development of florid angiomatosis. The vascular changes which have been described in association with pregnancy, the postpartum state and the use of oral contraceptives are quite distinct from the histopathological findings we have described here and consist primarily of intimal proliferation (Irey & Norris, 1973).

Endothelial proliferation is among the non-specific responses of vascular tissue to injury induced by any of a variety of mechanisms (Thorgeirsson & Robertson, 1978). However, the intense angiomatosis seen in this patient has not been reported in association with PAN. The mechanisms underlying the induction of angioendothelial proliferation in this case are by no means clear. In the setting of this immunologically mediated illness, angioendothelial proliferation might be mediated by the angiogenic factors elaborated by activated macrophages or by lymphocytes participating in an immune response (Folkman, 1982). Fibrin deposited as a result of active vasculitis might play a role in promoting angiogenesis by providing a scaffold for the laying down of new vessels. We also cannot address the question of whether this angioendothelial proliferation was limited to skin or occurred in other tissues. If the former were the case, this might be explained by local factors, including the possible participation of a previously-described, epidermally-derived angiogenic factor (Wolf & Harrison, 1973).

While histological re-examination during clinical remission would be interesting, this is precluded by the patient's maintenance on cyclophosphamide and prednisone, evidence of underlying superior vena cava obstruction, and a prolonged time required for wound healing following initial biopsy.

In addition to constituting a remarkable cutaneous reaction in a patient with PAN, the findings in this case underline the caution with which intense but benign angiomatous processes must be differentiated from the truly malignant, and reinforce the need for careful clinicopathological correlation.

## ACKNOWLEDGMENT

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# Granulomatous cheilitis and a positive Kveim test

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## SUMMARY

A female patient with a protracted history of multitudinary symptoms more recently developed granulomatous cheilitis (GC) and underwent extensive investigations. Her Kveim test was positive.

Oro-facial granulomatosis is a rare chronic granulomatous disorder involving the lips or the oral mucosa, or both, complicated by diffuse lymphoedema of varying extent in the lower half of the face.

There are case and group studies of the condition variously called granulomatous cheilitis and Melkersson Rosenthal syndrome (MRS) (Rook, 1979) and in only a minority of patients have aetiological associations been found; these are Crohn's disease (Verbov, 1973; Taylor & Smith, 1975; Wiesenfeld *et al.*, 1985), sarcoidosis (Wiesenfeld *et al.*, 1985), allergic responses to non-specific agents (Forman & Shuttleworth, 1956, Hornstein, 1960) and a familial pattern (Carr, 1966; Lygidakis & Trakamarakas, 1979; Levenson *et al.*, 1984). No overlap has been shown between this condition and other granulomatous diseases such as tuberculosis, leprosy and syphilis.

## CASE REPORT

A 43-year-old Caucasian woman with a long-standing history of multisystemic complaints including general malaise, aches and pains, abdominal pains, bloated feelings and difficulty in breathing, developed fluctuating lip and cheek swelling in late 1983. This became persistent in 1984 and progressively worse from February 1985. We saw her initially in 1984 and have undertaken her management subsequently.

On clinical examination, she was overweight and had a marked indurated lower facial swelling (Fig. 1). The remainder of her physical examination was normal and in particular there were no oral, regional or parotid gland changes and no cranial nerve deficits.

The following investigations were normal: Full blood count, ESR, biochemical profile,

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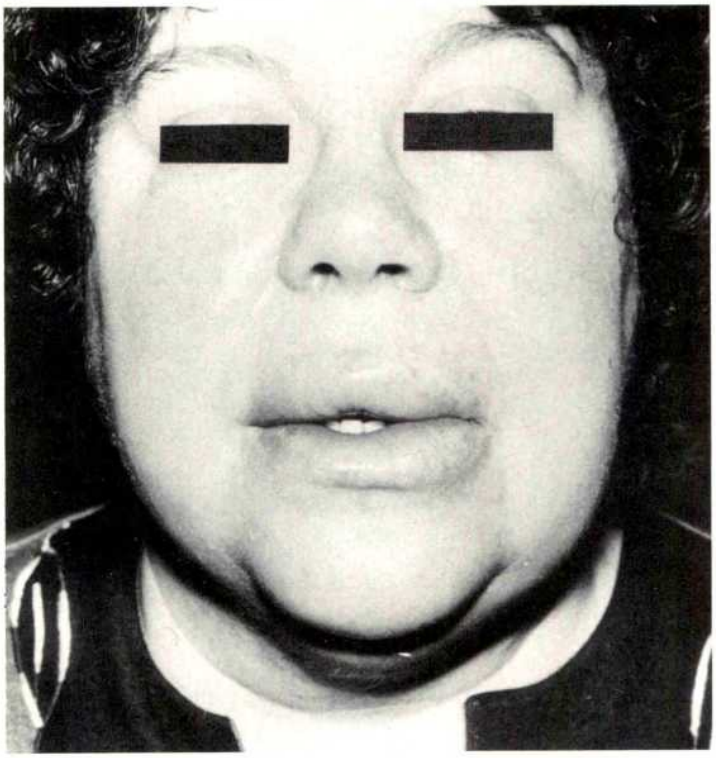

FIGURE 1. Clinical view of patient showing striking lip swelling.

protein electrophoresis and immunoglobulin assay. The urine was negative for Bence Jones and other proteins. Serum thyroxine, vitamin $B_{12}$ and folate were normal. Her $C_1$ esterase inhibitor was normal and challenge with food additives and azo-dyes precipitated no reactions.

Histology of a skin biopsy from the upper lip showed superficial and deep dermal non-caseating granulomata (Fig. 2) with no evidence of micro-organisms or foreign bodies. A Kveim test (K41/1/10) performed on the right forearm produced a well defined dermal epithelioid granulomatous response.

In 1985, the patient was admitted to hospital twice and further specific investigations were carried out in order to exclude an underlying systemic disorder. We were unable to confirm a diagnosis of systemic sarcoidosis having obtained normal chest and hand X-rays, normal ECG, EEG and respiratory function tests. The serum angiotensin converting enzyme remained within normal limits. A Mantoux test 1/1000 was negative. Two long-standing nodules on the left leg were excised and histologically proven to be histiocytomas. She was seen by an ophthalmologist and was found to have no eye abnormality, and a conjunctival biopsy was considered unnecessary.

Regarding a possible diagnosis of Crohn's disease, a barium enema, barium meal and follow-through were completely normal, and biopsies from jejunal and rectal mucosae yielded normal histology. Tests for faecal occult blood were repeatedly negative. Other serological tests for syphilis, R.A. latex and ANF were also negative.

Introducing an elimination diet made no clinical difference in her condition, nor did

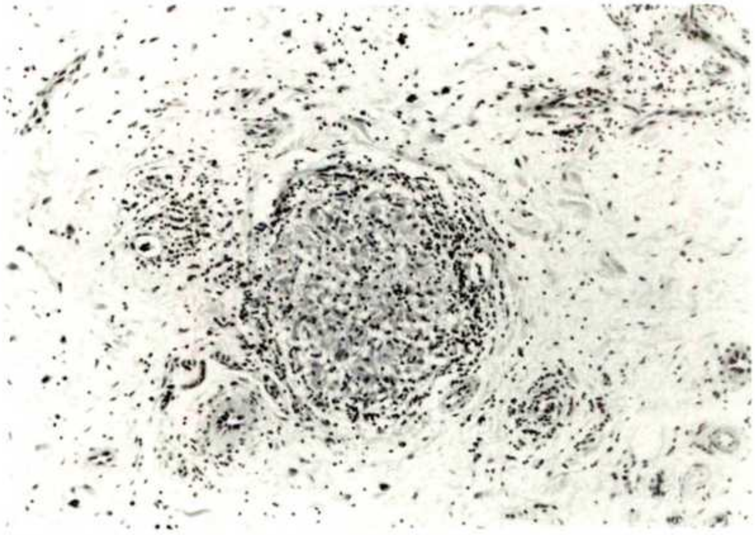

FIGURE 2. Biopsy of lip area illustrating epithelial cell non-caseating granuloma.

treatment with systemic steroids or salazopyrine; her facial swelling enlarged and advanced upwards.

## DISCUSSION

Known causes of oro-facial granulomatosis can be diagnosed with relative ease; however, the rest, that account for the majority of cases, yield negative results on extensive investigation. We were unable to discover a systemic disorder in our patient and can only speculate on the significance of a positive Kveim test.

Patients similar to ours on whom Kveim tests have been performed gave negative results (Rhodes & Stirling, 1965, Carr, 1974, Lindelöf, Eklund & Lideu, 1985) except in two out of a large series (published and unpublished), both of whom had co-existing cutaneous sarcoid lesions (Wiesenfeld *et al.*, 1985). Another patient of ours with Melkersson Rosenthal syndrome of 6 years' duration also had a negative Kveim test. A Kveim test is generally specific for sarcoidosis (Sharma, 1984); however, cross-reactivity between sarcoidosis and Crohn's disease occurs (Mitchell *et al.*, 1970), and other conditions reported by Israel and Goldstein (1971) to produce positive responses are tuberculous lymphadenitis, non-specific lymphadenitis and lymphoma. None of these are of immediate relevance in our patient unless overt manifestation of systemic disease appears in the future, as a case of granulomatous cheilitis has been reported to predate the onset of Crohn's disease by several years (Carr, 1974).

In view of the fact that the rate of Kveim reactivity is low among patients in whom the manifestation of sarcoidosis is confined to a single organ (Bradstreet, Gighero & Mitchell, 1976), and that the reactivity generally decreases sharply with the passage of time (Mikhil & Mitchell, 1970), a positive Kveim response obtained at an early stage of this disease and a negative Mantoux test, perhaps due to cutaneous anergy, strengthen the possibility that we are dealing with an atypical cutaneous sarcoidosis. Also, recognizing the limitations of Kveim test reactivity and taking into consideration the occasional spontaneous remission of this condition, we suggest that more cases of oro-facial granulomatosis may have eluded a diagnosis of cutaneous sarcoidosis in the past.

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# A case of multicentric reticulohistiocytosis: uncommon clinical signs and a report of T-cell marker characteristics

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## SUMMARY

We describe a patient with multicentric reticulohistiocytosis (MR) who demonstrated Koebner's phenomenon and had unusually large skin nodules. OKT3 and OKT4 lymphocyte markers were demonstrated on histiocyte membranes, which have not been previously reported in this disease.

## CASE REPORT

A 44-year-old Caucasian metallurgist presented to the Department of Rheumatology. He reported that over the past year he had developed pain in his knees which was followed after a few days by swelling. Over the following 2 weeks he had developed pain, stiffness and swelling of the metacarpo-phalangeal joints of both hands. He took a short course of a non-steroidal, anti-inflammatory agent with no benefit. His arthritis gradually settled over a few weeks. Six months later, after taking tetracycline for a sore throat, he had developed skin nodules. These were on his hands, elbows shoulders and pelvic area, and he had observed that they occurred at sites of pressure. At this time, the pain and swelling in his hands and knees had returned. His arthritis persisted continuously and on presentation it was becoming progressively disabling. He had, however, managed up to this point to avoid any absence from work. He also noted a weight loss of 16·5 kg over the year. He gave no past history of neoplasia, endocrinopathy or family history of arthritis or skin disease.

On examination he was cachexic with obvious loss of weight and had a mild pyrexia of 37·5°C. He had no significant lymphadenopathy. Examination of the skin showed erythematous nodules on the finger pulps which were acutely tender (Fig. 1). He had nail fold capillary dilatation and nodules from 3 mm to 15 mm on his fingers and elbows, with larger yellowish and dusky red tuberose lesions over his shoulders and pelvic area (Fig. 2). He also had nodules on the bridge of the nose at the site of pressure from his spectacles and around the thumb and first finger which

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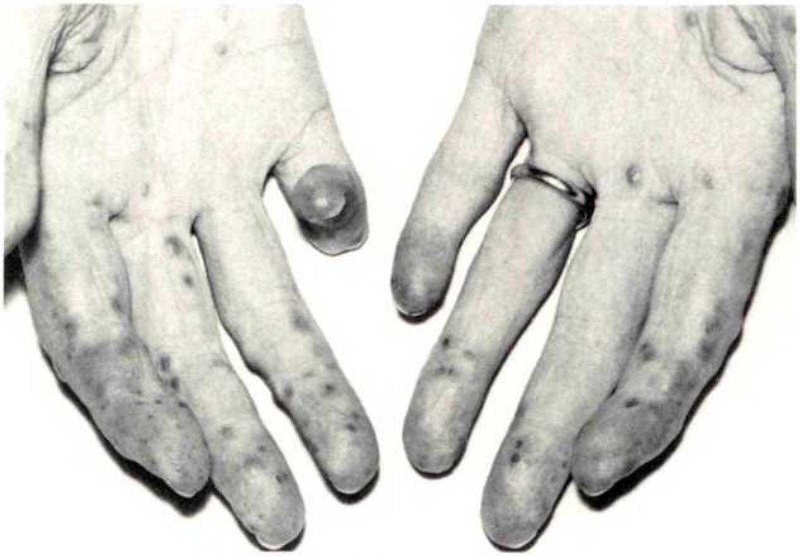

FIGURE 1. Nodules on the fingers.

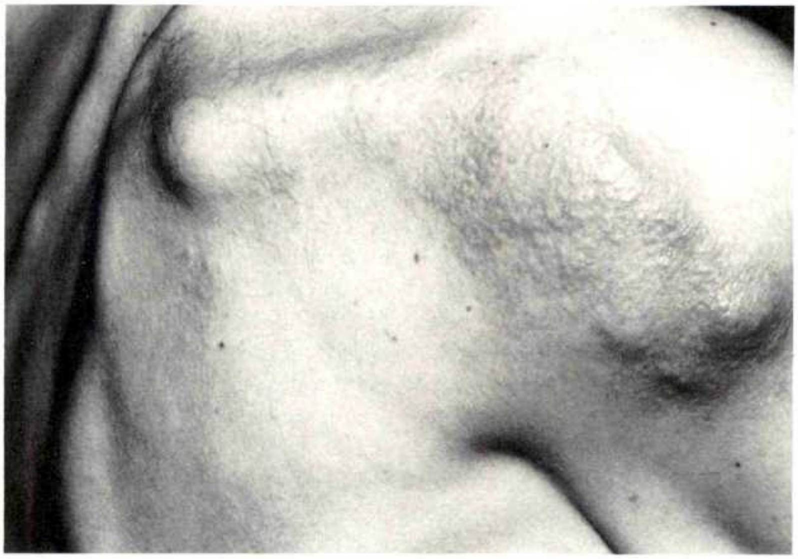

FIGURE 2. Tuberose lesion on the shoulder.

had appeared after unscrewing a tight jar. Examination of the joints showed him to have swollen and tender metacarpo-phalangeal joints and wrists. The knees were swollen with a positive patellar tap and he had fixed flexion deformities of 15 degrees bilaterally, flexion was otherwise full. Other joints appeared normal.

*Investigations*

A full blood count revealed haemoglobin 11 g/dl, white cell count $7\cdot6 \times 10^9$/l with a normal differential count, and platelets elevated at 570 × $10^9$/l, and he had an ESR of 66 mm/h. Autoantibodies, including rheumatoid arthritis haemaglutination test were negative. Serum IgG and IgA were slightly elevated. IgM was normal. Other blood tests including thyroid function tests, complement and lipids were normal. Repeated blood, throat and urine cultures were all sterile. Intradermal tuberculin testing at 1:1000 was weakly positive. X-rays showed peri-articular erosions. Aspiration of the knee produced only 2 ml of clear straw-coloured fluid which was sterile on culture.

### *Histology and immunocytochemistry*

Biopsies of nodules from the elbow and back were taken and sent fresh for analysis. Tissue was prepared for electron microscopy following gluteraldehyde fixation. Samples were also snap frozen at −70°C prior to cutting frozen sections for use with a standard peroxidase anti-peroxidase techniques, with appropriate controls (Taylor, 1978). The remaining tissue was fixed in 10% buffered formaldehyde, post-fixed in mercuric chloride prior to paraffin wax embedding, cutting and staining using conventional techniques.

Light microscopy showed disruption of dermal collagen by an infiltrate of large mononuclear cells interspersed with multinucleate giant cells containing ground glass cytoplasm (Fig. 3). The giant cells contained small amounts of neutral mucin (PAS positive, diastase resistant). No mitoses were seen. There were only a few evenly dispersed lymphocytes. These are the accepted features of multicentric reticulohistiocytosis.

Electron microscopy showed mononuclear cells and multinucleated cells with predominantly rounded nuclei and with cytoplasm rich in organelles. Lysosomes of varying sizes and some lipid were seen in both types of cell and complex interdigitation was seen between neighbouring cell membranes. Collagen was present between some cells but did not appear to be formed by the cells (Fig. 4). These were similar in appearance to ordinary histiocytes. Langerhans granules (Birbeck granules) were searched for extensively but were not found.

Immunocytochemistry for S100, neurone specific enolase, muramidase, α-1-antitrypsin

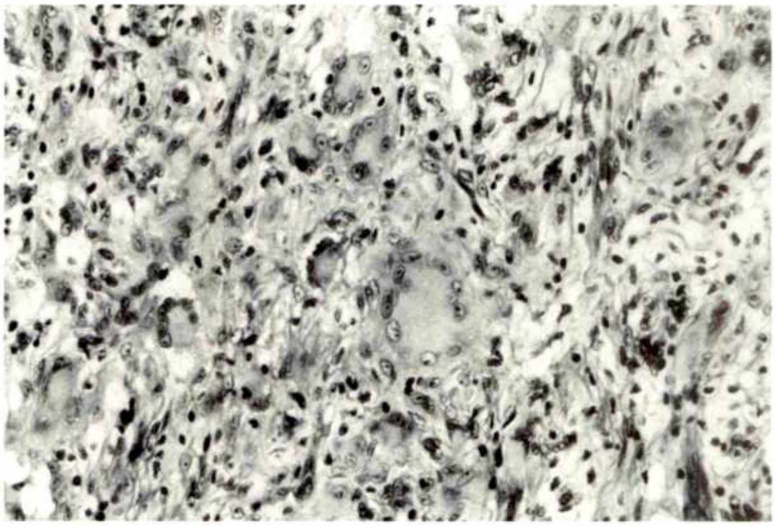

FIGURE 3. Dermal collagen infiltrated by mononuclear and multinuclear cells with ground glass cytoplasm (centre) (H & E; original × 280).

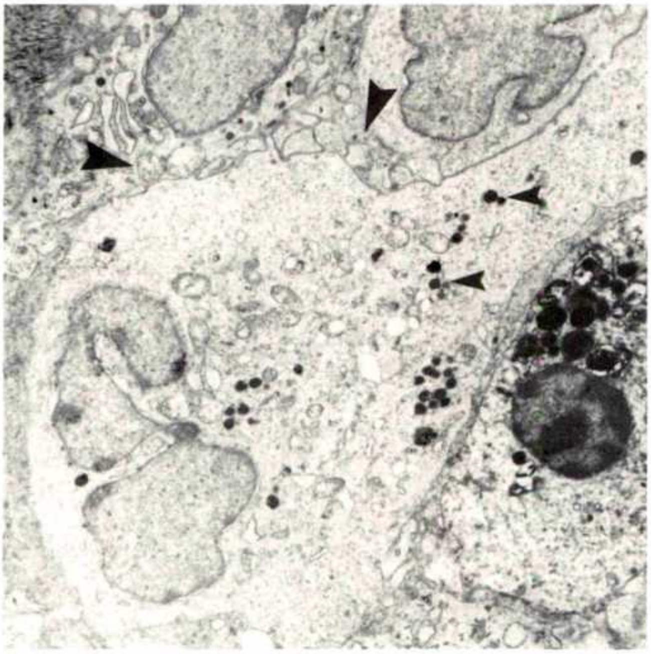

FIGURE 4. Elongated cell with interdigitation of the membrane with adjacent cells (large arrows) and lysosomes in the cytoplasm (small arrows) (electron microscopy; original × 5000).

TABLE 1. Results of immunocytochemistry

| Marker | Specificities | Reaction with MR cells |
|---|---|---|
| S100 | Langerhans cells melanocytes and neuroectoderm | ± |
| Neurone specific enolase | Langerhans cells Merkel cells, interdigitating reticulum cells, neural tissue | – |
| α-1-Antitrypsin (A1AT) | Phagocytes | + |
| Muramidase | Phagocytes | + |
| OKT3 | Pan-T-cell | + |
| OKT4 | T helper cells | + |
| OKT6 | Thymocytes, Langerhans cells interdigitating reticulum cells | ± |
| OKT8 | T suppressor cells and cytotoxic cells | – |
| OKM1 | Macrophages | + |
| MT1 | T cells | + |
| MB1 | B cells | – |

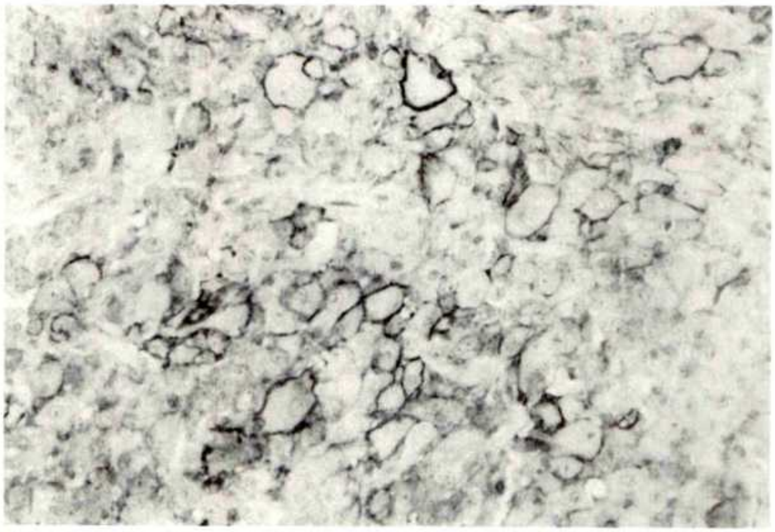

FIGURE 5. Positive membrane staining using OKT4.

(A1AT), OKT3, OKT4, OKT6, OKT8 and OKM1 was performed on frozen sections, and MT1 and MB1 on fixed tissue (Table 1). The large mononuclear cells and multinucleate cells showed cytoplasmic staining for the histiocytic markers, muramidase, A1AT and OKM1. There was no staining with neurone specific enolase and only a very few mononuclear cells stained with the Langerhans cell and interdigitating reticulum cell markers, S100 and OKT6. The cell membranes of the mononuclear and multinuclear cells stained with OKT3 and OKT4 (Fig. 5) but were negative to OKT8. Amongst the dispersed lymphocytes a moderate number were OKT8 positive, MT1 was positive in the large cells but MB1 was negative.

## DISCUSSION

Less than 100 cases of MR have been reported in the literature since it was first described as a systemic disease by Weber and Freudenthal (1937). The prognosis, based on the series of 33 patients described by Barrow and Holubar (1969), appears to be relatively good if the patient survives the initial onset; stabilization occuring after approximately 8 years of active disease. Unfortunately, arthritis mutilans may be the disabling outcome. Our case showed many typical features when compared to Barrow and Holubar's series and the series of eight described by Catterall (1980). There was strong circumstantial evidence of Koebner's phenomenon as also described by Aldridge, Main and Daly (1984). Some of the skin nodules had the appearance of tumours as they were unusually large in this case. In our patient, polyarthritis occurred first, and this is the commonest first presenting symptom. The following signs have previously been reported in MR but were not present in our patient: tendon sheath swelling, wrist ganglia, hyperextension of joints, fractures, mucosal nodules, generalised erythema, xanthelasma, lymphadenopathy, hypertension, endocrinopathy, hyperlipidaemia and a history of neoplasia.

Since it was first described the pathogenesis has remained obscure. It has been considered to be primarily histiocytic, hence its name, although at least 12 synonyms that appear in the literature reveal some confusion. Ebner and Gebhart (1971) and Hashimoto and Pritzer (1973) suggested the Langerhans cell as the possible cell of origin, but we did not find characteristic granules or markers of this cell and Coode, Ridgway and Jones (1980) concur that Birbeck granules are not found in genuine cases of MR. The histopathology, immunocytochemistry and electron microscopy show either an infiltrate which is of primarily histiocytic cells manifesting the T-cell markers OKT3 and OKT4, or a population of unusual T cells which reveal histiocytic organelles, enzymes and markers. Previous reports have not indicated the results of T-cell marker studies and it is difficult therefore to assess the significance here. Recently, Isaacson *et al.* (1985) created a similar quandary when finding T-cell markers on tumour cells of the previously defined histiocytic lymphoma complicating coeliac disease; these cells contained lysosomes, the cytoplasmic macrophage marker Leu M3 (equivalent to OKM1), and phagocytic enzymes. Also, Adams *et al.* (1984) found both T-cell and macrophage markers on atypical pale staining cells in a case of mycosis fungoides using similar markers. Further cases of this rare disease must be subject to T-cell marker labelling for further substantiation in order to establish the possible overlap between T cells and cells with phagocytic function.

## ACKNOWLEDGMENTS

We would like to thank Dr I.Griffiths and Dr C.J.Stevenson for allowing us to report their patient.

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# Generalized pustular psoriasis precipitated by trazodone in the treatment of depression

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## SUMMARY

Severe psoriasis is commonly associated with depression and may be its cause. The hazards of treating such patients with lithium are well known (Lazarus & Gilgor, 1979), but other drugs may also destabilize psoriasis. We report a patient in whom the anti-depressant trazodone hydrochloride (Molipaxin®, Roussel), a serotonin antagonist, provoked generalized pustular psoriasis (GPP).

## CASE REPORT

A 37-year-old Caucasian man had suffered from stable plaque psoriasis for 19 years. During the previous 3 years he had been treated with several intermittent short courses of weekly oral methotrexate and small quantities of diluted clobetasol proprionate (Dermovate®, Glaxo). There was no family history of psoriasis.

As his psoriasis gradually worsened, he became depressed and began treatment with trazodone hydrochloride. After 17 days, his psoriasis rapidly worsened with skin tenderness and fever and he was referred as an emergency to the Skin Department of the London Hospital.

Examination on admission revealed generalized pustular psoriasis on a base of nearly universal erythroderma with oedema of the skin at dependent sites and areas of exfoliation and micropustulation. Oral examination revealed a geographic tongue. He was febrile at 38·5°C. Urinalysis was normal.

Laboratory investigations showed haemoglobin 13·5 g/dl, white blood cells 10·6 × $10^9$/l, 57% neutrophils, 28% lymphocytes; renal and hepatic function tests and serum $Ca^{++}$ were normal.

Trazodone was stopped and he was treated conservatively with bland local applications. The fever and erythroderma settled. To maintain treatment of his depression, trazodone HCl 50 mg was recommenced 7 days later; only a single dose was given as after 14 h he became unwell and febrile (39·8°C), with a fresh erythroderma and pustulation on the left wrist. Investigations revealed a leukocytosis 16·5 × $10^9$/l with an absolute lymphopenia 0·82 × $10^9$/l. Therapy with methotrexate controlled this erythrodermic phase promptly. Methotrexate was reduced

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gradually and discontinued after 11 weeks with no relapse of pustular psoriasis, although plaques returned on the extensor surfaces. At no time during the course of his disease were there signs of cutaneous atrophy to suggest over-use of topical steroids.

## DISCUSSION

The course of events in this case strongly suggest trazodone as the cause of GPP. Gross topical exposure to very potent chlorinated corticosteroids can precipitate GPP, as can their withdrawal (Boxley, Dawber & Summerley, 1975). Our patient had not used sufficient quantities of potent corticosteroids for this to be a serious possibility. He had received no other oral drugs.

This report emphasizes the need for caution when planning treatment of the psoriatic with depression. Methotrexate can itself cause depression but this lifts rapidly when the drug is stopped (Baker, 1970). However, it was not responsible in this case as it had not been taken for several months prior to the episode. Lithium, which is used in the treatment of depressive psychoses, aggravates psoriasis (Lazarus & Gilgor, 1979) and may precipitate GPP, possibly by interfering with the cyclic nucleotide axis (Lowe & Ridgway, 1978). Other drugs which have precipitated GPP include salicylates, iodide, phenyl- and oxyphen-butazone, progesterone and propranolol (Baker, 1986).

Propranolol, like lithium, may interfere with cyclic AMP activity, and oxyphen-butazone may disturb the arachidonic acid cascade. Both biochemical mechanisms may be affected in psoriasis but it is not known whether these changes are primary or secondary. The precipitation of GPP by a serotonin antagonist suggests that an alternative mechanism may be involved.

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# Papular and nodular mucinosis associated with systemic lupus erythematosus

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## SUMMARY

A patient is described in whom a papulo-nodular eruption occurred in association with systemic lupus erythematosus. Histopathology disclosed a diffuse deposition of mucin, mainly hyaluronic acid, in the dermis. This, and the 10 similar cases which have been reported in the literature, belong to a distinct clinico-pathological entity for which we propose the eponym of papular and nodular mucinosis of Gold, after the first observer to report it.

Dermal mucinosis is a frequent histological finding in both discoid lupus erythematosus (DLE) and systemic lupus erythematosus (SLE) (Prunieras & Montgomery, 1956; Reed, Clark & Mihm, 1973; Weigand, Burgdorf & Gregg, 1981), but rarely does it produce clinically apparent manifestations. A distinct papular eruption characterized by mucinous dermal deposits has been described by Gold (1954) in two SLE patients, and has since been confirmed in eight further reports.

We describe here a typical case of this manifestation.

## CASE REPORT

A 24-year-old woman was seen because of a rash affecting her face, neck and arms. She reported that the rash had occurred after sun exposure one year earlier and had waned spontaneously. The following summer, after sunbathing, the rash recurred, requiring oral steroid treatment with which the patient did not comply very well. She also complained of low fever, fatigue, diffuse hair loss and arthralgia in her fingers.

On examination, her cheeks, nose and forehead were diffusely erythematous with a violaceous hue; erythema and keratotic scarring lesions were present on the extensor surfaces of her hands and forearms. Periungual telangiectasia and palmo-plantar erythrosis were also noted. Her hair came out easily when pulled gently. Her hand joints were swollen and tender.

Some indolent, skin-coloured papulo-nodules 5–15 mm diameter were present on her back,

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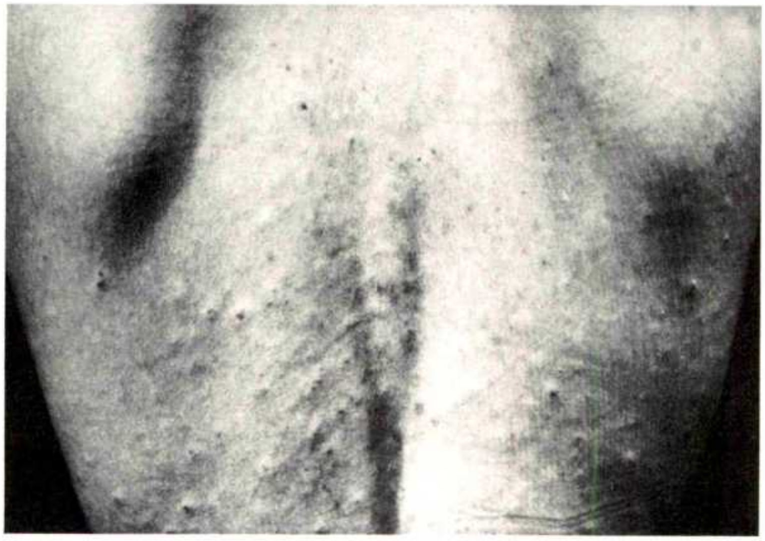

FIGURE 1. Papulo-nodular lesions on the back, giving a 'lumpy' appearance to the skin.

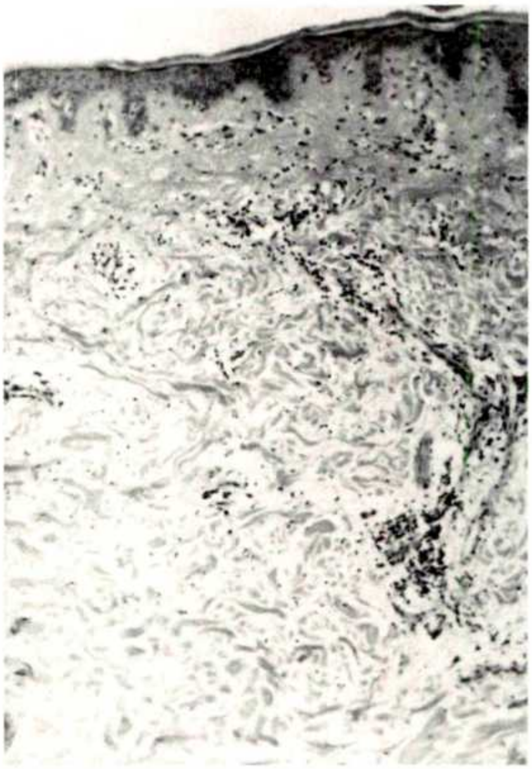

FIGURE 2. Collagen bundles are separated by mucin deposition. A slight lymphocytic infiltration surrounds small vessels (H & E).

chest and arms (Fig. 1). Some of these showed a reddish central depression. The nodules were more readily seen by tangential illumination giving a 'lumpy' appearance to the skin.

Laboratory tests revealed an elevated ESR, 41 mm/h; hypochromic anaemia ($3{\cdot}47 \times 10^6$ red cells/ml, haemoglobin 10 g/dl); leukopenia ($3{\cdot}62 \times 10^3$ WBC/ml); hypergammaglobulinemia without paraproteinemia; slightly increased levels of circulating immunocomplexes (19%, conglutinin method; normal, 0–17%), and reduced complement activity (CH50 420 haemolytic units, normal > 800). Anti-nuclear antibodies were positive at 1:640 with a speckled pattern. Anti-ENA antibodies were absent. Farr assay was positive (112%, normal 10–20%), but the Crithidia luciliae immunofluorescence test was negative.

Urinalysis, BUN, serum creatinine, and creatinine clearance were normal. T3, T4 and TSH were normal.

Histopathology of an erythematous patch on the dorsum of one hand showed slight liquefaction degeneration of the basal layer, incontinence of pigment in the upper dermis and a diffuse fibrinoid degeneration and oedema in the middle dermis. Direct immunofluorescence (DIF) performed on the same lesion disclosed granular deposits of IgG, IgM and C3 along the dermo-epidermal junction. Histopathology of a nodular lesion on the neck showed a slight separation of collagen bundles suggestive of dermal oedema (Fig. 2). PAS stain was negative; Alcian blue stain at pH 4·0 was intensely positive, whereas at pH 0·4 it was completely negative, indicating a mucinous deposit composed of hyaluronic acid. A perivascular infiltrate was present in the upper dermis. No typical features of SLE were seen. DIF showed linear deposits of IgG and granular deposits of IgM along the dermo-epidermal junction. In addition, C3 deposits were seen in the deep vessel walls.

Clinical and laboratory evidence led to the diagnosis of SLE with an unusual papulo-nodular eruption related to a diffuse dermal mucinosis.

The patient was followed up for 3 months. The lupus rash cleared with 500 mg/day chloroquine and avoidance of sun exposure. The papulo-nodular lesions greatly improved, at present affecting only the upper back, and the central erythematous depressions have disappeared.

## DISCUSSION

The original description of this singular eruption dates back to 1954 and was due to Gold who found it in two SLE patients and stressed its correlation with the lupus erythematosus (LE) activity.

De Graciansky *et al.* (1956) reported a third case in an SLE patient, and this was again regarded as a sign of the worsening of the main disease.

Twenty-one years later, Salamon, Radovanovic and Lazovic (1977) described two new patients whom they diagnosed as having lichen myxedematosus associated with SLE and DLE respectively. The latter case is apparently the only one in a patient with DLE, but in the absence of any serology the diagnosis should be regarded as simply morphological. The mucinous lesions antedated the manifestations of LE.

A further patient with papulo-nodular mucinosis and SLE was reported by Gammon *et al.* (1978), who suggested that mucinosis was secondary to SLE and not a coincidental occurrence of two diseases. They stressed again that mucinous lesions varied with LE activity.

Kennedy, Gold and Moss (1979) described an SLE patient with an unusual papular eruption due to dermal oedema. Mucin was not detected, but ultrastructural findings suggested that this case was similar to Gold's original cases. Moulin *et al.* (1980) reported a photosensitive papulo-

TABLE 1. Reported cases of papular and nodular mucinosis (Gold)

| Year | Reported by | Sex | Age (years) | Diagnosis | Visceral involvement | ANA* | DIF | Comments |
|---|---|---|---|---|---|---|---|---|
| 1954 | Gold | F | 47 | SLE | Kidney | Not done | Not done | Death in 6 months |
|  | Gold | F | 41 | SLE | Joints | Not done | Not done | Varied with lupus activity |
| 1956 | De Graciansky *et al.* | M | 26 | SLE | Joints, kidney | Not done | Not done | Varied with lupus activity |
| 1977 | Salamon *et al.* | M | 20 | SLE | Joints | Not done | Not contributory | Antedated lupus |
|  | Salamon *et al.* | M | 30 | DLE | None | Not done | Not done | Antedated lupus |
| 1978 | Gammon *et al.* | M | 43 | SLE | Kidney, CNS | 1/120 hom. | IgG gran. | Varied with lupus activity |
| 1979 | Kennedy *et al.* | F | 20 | SLE | Joints, kidney | 1/80** | IgM** | Persisted after therapy |
| 1980 | Moulin *et al.* | F | 56 | SLE | Joints | 1/4096 hom. | IgG gran. | Antedated SLE, photosensitivity |
| 1982 | Revier *et al.* | F | 34 | SLE | Joints, kidney | 1/1280** | IgM, IgG, IgA l. | Antedated lupus |
| 1984 | Fowler & Callen | F | 48 | SLE | Joints, kidney, heart, serous membranes | 1/320 hom. | Not done | Persisted after therapy |
| 1986 | Our case | F | 24 | SLE | Joints | 1/640 sp. | IgG l. IgM gran. | Varied with lupus activity |

* Antinuclear antibody patterns; ** not specified; hom., homogeneous; sp., speckled; gran., granular; l., linear.

nodular mucinosis which had occurred 3 years prior to any clinical or serological evidence of SLE.

Revier *et al.* (1982) reported a further case in which the papulo-nodules antedated SLE by 2 years and suggested that the concurrence of the two diseases was not fortuitous. Both SLE manifestations and cutaneous mucinosis cleared with oral steroids.

Finally, Fowler and Callen (1984) described a papulo-nodular mucinosis in an SLE patient that persisted despite anti-malarial and corticosteroid treatments.

Our patient is quite similar to the previously reported cases. She undoubtedly has SLE, and her papulo-nodular eruption proved to be due to a diffuse dermal deposition of mucin, mainly hyaluronic acid. Like all the other patients described in the literature, she had neither paraproteinemia nor dysthyroidism.

We cannot say whether mucinosis preceded SLE in our patient; certainly, it varied according to LE activity since it cleared, though more slowly, with antimalarial treatment. Despite the history of sun exposure at the beginning of her illness, our patient exhibited the mucinous nodules on usually sun-protected areas. It is therefore unlikely that photosensitivity has any pathogenetic role, as was the case in the patient described by Moulin *et al.* (1980).

Papulo-nodular mucinosis of Gold appears to be a distinct, though unusual, cutaneous manifestation occurring in patients with SLE, that is not mentioned among the characteristic LE manifestations in the major dermatology textbooks. It presents as indolent flesh coloured papulo-nodules on the neck, trunk and upper limbs that give a 'lumpy' appearance to the skin and are due to a diffuse mucinous deposit in the dermis.

It differs from the diffuse mucinosis that can be found occasionally in the biopsies of DLE (Weigand *et al.*, 1981) and SLE lesions (Prunieras & Montgomery, 1956; Lamberts & Lynch, 1981) in that it occurs in areas free from specific LE lesions and produces clinically distinct manifestations.

In 90% of cases, the prevalent clinical features of the associated SLE involve joints and kidneys. In one-third of cases, mucinosis seems to be related to LE activity, although in some patients it persisted despite antimalarial and oral corticosteroid treatment (Table 1). Patients have been studied incompletely from the immunological point of view, mostly because some of them were seen many years ago. In those cases which have been studied, DIF showed a typical IgG or IgM lupus band, or both, and antinuclear antibodies and anti-double-stranded DNA antibodies were present at significant titres.

Although its pathogenesis remains obscure, clinical recognition of papular and nodular mucinosis of Gold is important because in one-third of cases it precedes both clinical and serological evidence of SLE by a period of up to several years.

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## First blast of the trumpet against the monstrous regiment of scientists (with apologies to John Knox)

MADAM, The letter by Haigh *et al.* (1985) leading to verbal skirmishing between themselves and Gaylarde (1986) and Gibson and Kirsch (1986) represents a further episode in the ongoing skin blanching test saga. Your readers, presented with Gaylarde's 'The human skin blanching test—use and abuse', must be in fear of an Editorial 'Whither the skin vasoconstriction test?'.

When I described the test in 1962 (McKenzie, 1962)—was it nearly a quarter of a century ago?—it was simple and reproducible. It worked in the humid warmth of Cleveland, Ohio, it gave consistent results in the claustrophobic and vertical confines of St John's, Leicester Square, while a few miles west of London it briskly, overnight, and with a minimum of fuss, sorted out more than a score of betamethasone esters and led to the introduction into clinical practice of several topical steroids which are still in everyday use (McKenzie & Atkinson, 1964). At that time I felt I was in danger of inducing boredom in my audience and withdrew from further active involvement in the vasoconstrictor test. Others have not had the same reticence. For some it has been a major preoccupation and it has produced diversion for and papers from many clinical and pharmacological departments. The original description has undergone a variety of culinary manoeuvres, garnishing from time to time, occasional refining and, most frequently of all, réchauffage.

Science will have its way, so that 'area under the curve', reflectance methods for measuring skin colour, and multiple reading techniques have been developed and each variation has been propounded as though it was University Grants Committee inspired. These investigators must have left many square metres of vasoconstricted skin in their wake, and eventually there may be some practical results from their tortuous and ever more complex methodology.

Authors are encouraged to murder their darlings and so it must be for investigators—but when complete strangers act as executioners, then paternal instincts are aroused. Petulant foot stamping such as '. . . the blanching test should no longer be accepted as adequate . . .' (Gaylarde, 1986) and '. . . we do not advocate the use of the human skin blanching assay' (Haigh *et al.*, 1986) may be acceptable in the fever of intellectual argument on paper but could be regarded as a less than fair critique of the test which I described with an all or none result being read at approximately 16 hours. If there are faults in the test then perhaps they lie in that unpredictable membrane, skin, which for a variety of reasons may be as temperamental as its increasingly fastidious researchers. All in all, in a light-hearted, uncontentious and biased way, I think that the test which I described had the virtue of originality and is still rather a good one.

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## Acro-osteolysis and the nail unit

MADAM, The article by Baran and Juhlin (*British Journal of Dermatology*, 1986, **114**, 371) on bone-dependent nail formation brings to mind the entity of acro-osteolysis and its effect on the nail unit. In this disorder, there is destruction that affects the distal phalanx and sometimes the middle and proximal phalanges as well. As a consequence of this chain of events, abnormalities of one or more components of the nail unit always follow.

Acro-osteolysis may occur secondary to a broad spectrum of conditions (Kemp, Valinka & Schumacher, 1986). These include arthritis (all types), hyperparathyroidism, vascular and traumatic phenomena, and neuropathic, neuroplastic, dermatological and infectious factors. There is also a large idiopathic and congenital category that must be considered.

Two cases are presented here to illustrate the relationship of bone and the nail.

Case 1 was a 34-year-old Caucasian male who had had arthritis associated with primary systemic sclerosis for 10 years. An X-ray of the second and third fingers revealed extensive destruction of the distal phalanges as well as joint involvement (Fig. 1). The nail unit of the second digit revealed a shortened nail plate, the transverse diameter of which exceeded its longitudinal diameter (racket nail) with subungual hyperkeratosis and onycholysis. The nail unit of the third digit was almost totally destroyed except for a small residual spicule (Fig. 2).

Case 2 was a 29-year-old Caucasian male whose fingers were burned as a consequence of an explosion that traumatized not only the skin but also the underlying bone. X-rays showed destruction of distal and middle phalanges with obliteration of the distal interphalangeal joint space (Fig. 3). The nail unit displayed transverse overcurvature of the nail plate with compression of the underlying nail bed (pincer nail), which caused the patient considerable pain. There was also onycholysis and subungual hyperkeratosis (Fig. 4).

Acro-osteolysis in association with connective tissue disorders is a well known phenomenon (Bunch, O'Duffy & McLeod, 1976). The inevitable consequence of this scenario and its ultimate effect on the nail unit, however, is less well recognized and merits further emphasis. Likewise, the occurrence of traumatic acro-osteolysis (Resnick & Niwayama, 1981), with concomitant onychodystrophy, should also be mentioned to alert the clinician to recognize the close bond between nail and bone.

Dermatologists, in general, are often particularly remiss in ordering X-rays when evaluating their patients who present with disorders of the nail unit. Neoplasms, infections, inflammations, and hereditary conditions of bone and joint may produce a change in the nail as their first clinical manifestation. Therefore, it behooves us to order X-rays in many of these patients, so as not to overlook important underlying pathology.

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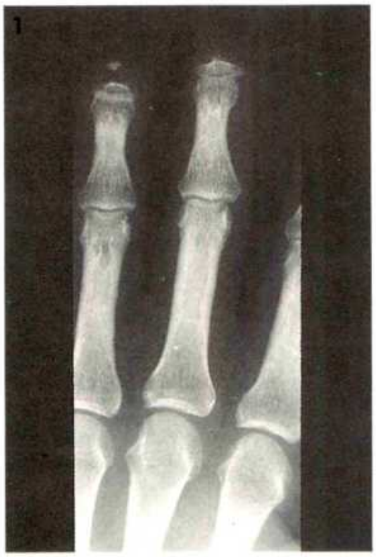

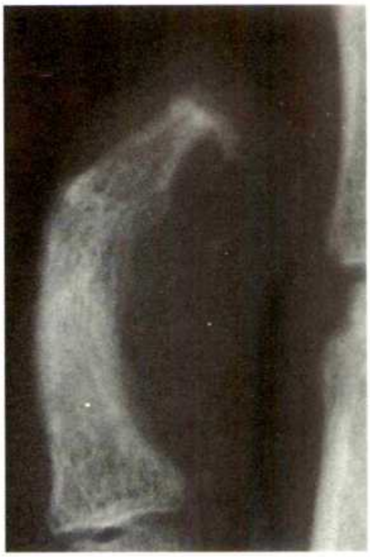

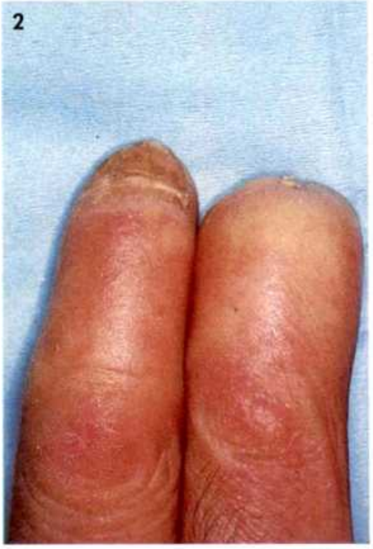

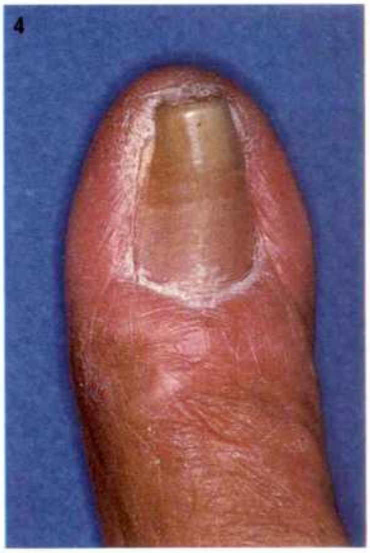

FIGURE 1. Case 1. X-ray of the right second and third fingers. There is extensive acro-osteolysis of the distal phalanges of both digits with obliteration of the distal interphalangeal joint space.

FIGURE 2. Case 1. Clinical picture of fingers shown in Figure 1. The second digit nail unit reveals a greater transverse than longitudinal diameter (racket nail) with subungual hyperkeratosis. The third digit nail unit is completely absent except for a small fragment of residual nail plate.

FIGURE 3. Case 2. X-ray of the right second finger. There is acro-osteolysis of the distal phalanx with spur formation and fusion of the distal interphalangeal joint space.

FIGURE 4. Case 2. Clinical picture of the finger shown in Figure 3. The second digit nail unit reveals transverse overcurvature of the nail plate distally (pincer nail) with subungual hyperkeratosis and acral onycholysis. The underlying distal nail bed and hyponychium is painfully compressed.

## Methotrexate for intractable benign familial chronic pemphigus

MADAM, We report the use of methotrexate to treat a man with intractable, widespread benign familial chronic pemphigus (BFCP). We have not found any other report of methotrexate being used to treat this disease.

A 50-year-old man presented with a 2-month exacerbation of BFCP. He had had BFCP since his mid-twenties and there was no apparent reason for the worsening of his disease. He was otherwise well and taking no systemic treatment. On examination he had plaques of BFCP around his neck, in and extending out of both axillae, and on the chest, abdomen and back. He was admitted to the ward and unsuccessfully treated over the next 3 months with topical steroids, topical and systemic antibiotics and anti-candidal agents, Grenz rays, iontophoresis and dapsone. At the end of this time, his rash was still too extensive to treat with skin grafts and too active for him to return to work. He was then treated empirically with 15 mg of intramuscular methotrexate per week. His BFCP started to improve within 2 weeks and was nearly clear one month later. He continued to receive methotrexate for 3 months and his skin remained clear. It was then stopped and 2 months later his BFCP reappeared in the axillae. His BFCP has been controlled by topical steroids alone during 2 years' follow-up.

It is possible that the administration of methotrexate coincided with a natural remission of this patients' disease. However, intractable BFCP is rare and we are unlikely to be able to repeat our observations for some time. Meanwhile, we hope that others may benefit from this observation.

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## Wallace's Line in serum sickness and Kawasaki disease

MADAM, Whatever the underlying reason for the existence of Wallace's Line, as discussed by Drs Rowland Payne and Branfoot (*British Journal of Dermatology*, 1986, **114,** 513), we would like to call attention to two other disorders in which cutaneous changes may be delimited sharply at the margin between the dorsal and plantar skin of the foot or dorsal and palmar skin of the hand. Lawley *et al.* (1984) described a striking and characteristic serpiginous erythematous and purpuric eruption on the hands and feet at the junction of palmar and plantar skin in eight of eleven patients with serum sickness. The photographs in their paper bear striking resemblance to the diagrams in Rowland Payne and Branfoot's letter.

During the 1979–1980 epidemic of Kawasaki disease in Massachusetts (Bell *et al.*, 1981), we observed a nearly identical configuration of 'scalloped erythema' on the feet and hands of several patients. Other features of the exanthem in the cases we observed, including distinctive transient micropustules, have been reported elsewhere (Rhodes *et al.*, 1981).

The observation of this configuration in one disease in which immune complexes are clearly involved (serum sickness), and in another in which they may play a role (Kawasaki disease), suggests not only that the anatomy of the lymphatic and vascular systems is important in how rashes localize, but also that the list of conditions in which Wallace's Line may be apparent has not yet reached its limit.

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A.R.RHODES
J.W.MELSKI

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*British Journal of Dermatology* (1986) **115**, 641–644.

# *Book Reviews*

**Optoelectronics in Medicine.** Proceedings of the 7th International Congress with the 2nd International Nd:YAG Laser Conference, LASER 85. Edited by W.WAIDELICH and P.KIEFHABER (1986) Berlin: Springer. Pp. 531. Price DM 108.

This volume of conference proceedings contains two sections. The first part consists of over 50 papers concerned with all aspects of current laser usage presented at the seventh biennial congress in Munich. Half of the papers are in German with no English abstract and only four (all from Germany) relate directly to dermatology. The first of these is a statistical analysis of 249 patients with port-wine stains treated with the argon laser and the second an analysis of 8 years of laser therapy with argon and carbon dioxide lasers. The third paper is an investigation of water cooling of the skin during argon laser therapy and the fourth a clinical paper concerned with the treatment of naevi with a combination of lasers and surgery. One third of the papers in this part of the proceedings are about laser biostimulation, reflecting the growing interest in this subject and the need to demonstrate in controlled trials any clinical usefulness for 'soft-lasers'.

The second half of the book consists of 51 papers on the Neodymium:YAG laser. All of these are in English but only three are of immediate relevance to dermatology. Two of these are from the Munich group, the first a brief general review of lasers in dermatology and the second a short clinical paper showing that the ND:YAG laser can be used in a similar way to the argon laser for the treatment of facial telangiectasia. The third paper, from Cincinatti, deals with Q-switched ND:YAG lasers which, as yet, are of limited value in dermatology.

As in previous years, the proceedings have been produced very quickly and editorial control seems to be slight. The preface comments that new ideas (in laser medicine) are developed with great enthusiasm and this certainly comes through in some of the papers. Laser medicine is being watched very closely as other clinicians see laser users redefining apparently established techniques in the light of new theories and research. The only way forward is a steady, scientifically-based advance, uninfluenced by talk of a billion pound market place for lasers in 1995. Commercial forces are becoming all too strong, but reliance on industry for funding puts undue pressure on research workers to make extravagant claims.

This book is probably one for the specialist laser user who will find many useful ideas and references. It is not a book to buy if one wants a balanced overview of the medical applications of this rapidly advancing technology.

N.P.J.WALKER

**Skin Problems in the Elderly.** Edited by L.FRY (1985) Edinburgh: Churchill Livingstone. Pp. 366. Price £38.00.

I looked forward to reading this book, expecting to be enlightened in my approach to diagnosis and treatment of skin disease in the elderly. This expectancy was enhanced by the introduction—'The diseases which strike old people, the symptoms and the signs which are induced, and the response to treatment are distinctive'. As I read on, a note of hesitancy was introduced—'Age associated differences are relative, not absolute', and 'Ageing begins pre-teens'.

I found the book to give little specific advice on diagnosis and treatment in what I consider to be the elderly. Indeed, much was allocated to disease rare in the aged population, e.g. lichen planus. The long-term side-effects of PUVA are discussed, but not the actual physical and psychological difficulties of giving an old person PUVA treatment. Can they manage to stand in an upright apparatus? Can they tolerate the heat and claustrophobia?

Those chapters dealing particularly with the ageing skin left me confused, with apparent contradictions on absorption, epidermal thickness, irritation, microcirculation and quantity of elastic fibres.

Many chapters, particularly those on pemphigus and pigmentary disorders, were a delight to read. It is a shame, though, that writing styles differ so much, from purely tabular to totally discussive; also, that the book gives the impression of being compiled in a hurry with frequent spelling and printing errors.

I feel that this book does not live up to my expectations of it dealing fully with those specific problems of the elderly, and as both dermatologists and geriatricians will have access to more complete texts, I find it difficult to advise either group to part with their £38.00 for this purchase.

J.ANDERSON

**Pathology of Unusual Malignant Cutaneous Tumours.** Edited by M.R.WICK (1985) New York: Marcel Dekker. Pp. 419. Price US$83.50.

This book is one of a series entitled 'Clinical and Biochemical Analysis', which includes such titles as *Ligand-Binder Assays: Labels and Analytical Strategies* and *Colorimetric and Fluorometric Analysis of Organic Compounds and Drugs*. This volume, however, deals with a subject of great importance to pathologists and clinicians having to manage patients with skin tumours. There is an excellent team of contributors and the subjects covered include sweat gland and pilar carcinomas, sebaceous carcinoma, primary cutaneous neuroendocrine carcinoma, vascular and non-vascular sarcomas, melanoma, lymphomas and examples of metastatic tumours from extracutaneous sites.

Benign tumours of adnexal structures are rarely a diagnostic problem for the trained pathologist. Malignant counterparts of these tumours, however, are much rarer and precise histological criteria for these malignant variants, as the authors point out, are often controversial and ill-defined. A wealth of information and references are provided on various sweat gland carcinomas including epidemiological, clinical, histological and immunohistological details. Attention is also drawn in this chapter to the increasingly recognized locally aggressive tumour that is so frequently mis-diagnosed by clinicians and pathologists alike: the microcystic adnexal carcinoma.

Wick and Scheithauer have provided a masterly review of the current knowledge relating to so-called primary neuroendocrine carcinoma of the skin, with interesting comments on possible histogenesis and a useful discussion of differential diagnosis. The chapter on vascular tumours is also very competently treated, although the authors (probably wisely) sit on the fence a little regarding the direction of vascular differentiation or derivation in Kaposi's sarcoma and angiosarcoma. They are, however, up-to-date in their views on the nature of angioendotheliomatosis and mention recent evidence to support the theory that this condition probably represents an 'angiotropic lymphoma' rather than a true vascular neoplasm.

The chapter on malignant melanoma I found a little disappointing. Although the important concepts of desmoplastic and neurotropic melanoma were briefly discussed, little mention was made of melanoma arising in congenital naevi, small cell melanoma and Richard Reed's concept of minimum deviation melanoma. I also found myself not entirely convinced of the value of separating off 'spindle cell melanoma' as an entity in its own right.

Although in the chapter on lymphoid neoplasms of the skin there is much useful information, there is little emphasis on differential diagnosis of these difficult tumours apart from a table indicating some pointers in separating benign from malignant lymphoid infiltrates. Another problem with this chapter is that the author puts forward the so-called Working Formulation Lymphoma classification as a pragmatic approach for everyday use. The lymphomas that one commonly encounters in the skin I find do not fit comfortably into this classification—but this may be personal prejudice!

Overall, this volume has much to commend it. It is up-to-date, and well illustrated with numerous histological, immunohistological and ultrastructural photomicrographs. There is much information here that I believe is quite unavailable in other textbooks and I heartily commend it to pathologists, oncologists and the dermatologist who wants to describe the next case in the world literature of trichochlamydocarcinoma!

N.P.SMITH

**Acquired Immuno Deficiency Syndrome. Progress in Allergy.** Vol. 37. Edited by E.KLEIN (1986) Basel: Karger. Pp. 398. Price SFr. 275.

This, the 37th volume in the series 'Progress in Allergy', is devoted to the subject of the Acquired Immuno Deficiency Syndrome. It is a brave man (or in this case a woman, Dr Eva Klein) who edits a book on AIDS;

such is the speed of progress in research into the subject that much of the material is inevitably out-of-date by the time it is published. The editor, however, has done an excellent job in selecting a number of experts to review many aspects of the disorder, which, of course, includes the basic immunological, virological, epidemiological and pathological data. She has also arranged for chapters on the Simian acquired immunodeficiency syndrome and feline leukaemia virus-induced feline AIDS to be included, as well as addressing in separate chapters the role that the Epstein Barr virus plays in lymphomas in homosexual men and the role cytomegalovirus plays in Kaposi's sarcoma and AIDS syndrome.

It is not easy to single out chapters for special praise or criticism as the all round standards are so high and the fields covered are so wide that few experts would agree with all that is written and few non-specialist doctors would have sufficient knowledge to proffer detailed criticism. All is well written and splendidly referenced to include most of the important primary material—indeed, the book contains 2400 up-to-date references.

The chapter on the histology of lymph nodes from ARC and AIDS patients patiently reviews a mass of confusing material (321 references) showing the many difficulties inherent in the histological diagnosis of PGL and AIDS from nodal biopsies. Worthy of special note is the chapter on AIDS in children—a sad, sad story within a sad story. The book concludes with an illuminating account of the immune defects that operate in leprosy. In short, a book for the AIDS worker and the library where it will be consulted by doctors practising in many specialities.

J.K.OATES

**Low Dose Methotrexate Therapy in Rheumatic Diseases.** Edited by R.RAU (1986) Basel: Karger. Pp. 268. Price £70.20.

This ninth volume of Annual Reviews of Rheumatology comprises the proceedings of a symposium on this subject held in Germany in 1984. To the dermatologist it is intriguing how the subject has come full circle since the report in 1951 by Gubner and colleagues on the treatment of rheumatoid arthritis with aminopterin. Their incidental observation on the response of one patient's psoriasis, taken up by Rees B.Rees and his colleagues in San Francisco, led to the greatest single advance in the management of psoriasis since dithranol. Yet rheumatological interest languished for 30 years until the reappraisal of methotrexate (MTX) in rheumatoid disease in the eighties. This renewed interest benefits from the huge legacy of knowledge on the pharmacokinetics and toxicity of MTX accumulated since 1950 for high dose therapy in oncology and low dose usage in psoriasis.

The book is comprehensive but with the usual unevenness of quality characteristic of published symposium proceedings. Heavy-weight contributions from Nyfors, who has made MTX his special interest for 20 years, Rau and Herborn, and Karger and Rau in Ratingen, and Wilke and colleagues at the Cleveland Clinic, form the backbone of the book, together with the results of a large American multi-centre controlled trial.

What does it all tell us? In fact, the answer was neatly summarized in a Lancet editorial (11 January 1986). MTX works in rheumatoid arthritis but at the price of a good deal of hepatic, pulmonary and bone marrow toxicity; no surprises for dermatologists there. Whether MTX is more effective than gold or penicillamine, whether it can arrest erosive joint deterioration and whether its risk:benefit ratio is acceptable, awaits determination.

H.BAKER

**Pathogenesis of Skin Disease.** Edited by B.H.THIERS and R.L.DOBSON (1986) New York: Churchill Livingstone. Pp. 642. Price £85.00.

This remarkable book will be welcomed by those dermatologists who look beyond the treatment of skin disorders and want to know the latest on their pathogenesis. Dr Thiers and Dr Dobson should be congratulated on their choice of subjects and authors. They have persuaded 65 well known contributors to write on 40 mostly well known groups of conditions. The approach is not a broad brush one and the information and references contained in each chapter are very comprehensive. At the time of writing, I can think of no worthy competitor which provides such up-to-date and accessible details on the whys and hows of the diseases which are our raison d'etre.

The chapters are broken up by well chosen and clear headings and the text is generally concise. At the end of many chapters there is a helpful section with a summary of what is known about the pathogenesis of the disease in question and sometimes a bold hypothesis on the aetiology which tries to link the findings. Many line diagrams and tables clarify difficult points and multifactorial interactions. The relatively few black and white clinical pictures simply signify that this is not, thank heavens, another book about the diagnosis and treatment of skin diseases and are a reminder that the editors have successfully coaxed their contributors to focus on pathogenesis. There are numerous references dating back to at least 1895 (contact dermatitis and epidermolysis bullosa) and up to 1984.

Although I have heard a number of these authors review their subjects at various recent meetings, I have learnt much more from reading their work in this book during 8 hours spent on the Edinburgh–London train than in 80 conference hours. Seen in this light, the book provides very good value for money but, for all that, it is one that decent departments will have to get regardless of the price.

J.A.A.Hunter

**Infectious Diseases in the Female Patient. (Clinical Perspectives in Obstetrics & Gynecology).** Edited by R.P.Galask and B.Larsen (1986) New York: Springer Verlag. Pp. 264. Price DM 224.

This volume is one of a series devoted to 'major clinical areas in the health care of women'. From the series title it is clear that this is woman in her obstetric gynaecological aspects, in the proper consideration of which doctors other than obstetricians and gynaecologists will often be involved. The authors, all from the U.S.A., come from a variety of appropriate disciplines. The book is well produced with a good index and abundant references.

The chapters aim to explain the basic scientific background of infections. Some deal with infections as such—e.g. of the lower or the upper genital tract, or post-operative problems; others discuss such concepts as bacterial and viral infection and immunity, the compromised host, antimicrobial therapy and bacterial resistance.

To produce a book of such multi-disciplinary aspiration is difficult but the aim has been well achieved. In its different way, each chapter clearly focuses on appropriate clinical considerations. Little of the content of the book is of direct relevance to the dermatologist, and much of what is so has been adequately covered in dermatological texts. However, the chapter by Stahl and Hill on the microflora of the female genital tract, and that by Eschenbach on lower genital tract infections, provide a wealth of clearly tabulated and analysed material not easily found elsewhere.

C.M.Ridley

*British Journal of Dermatology* (1986) **115**, 645–646.

# *News and Notices*

## British Association of Dermatologists

*The Dowling Travelling Fellowship 1987.* The Dowling Travelling Fellowship for 1986 has been awarded to Dr C.B.Archer. It has been agreed by the Executive Committee that the Fellowship should be awarded again in 1987. Applications are invited for the above Fellowship which is tenable for up to 2 years (value £10 000 p.a.). It is intended to help support a meritorious applicant who wishes to undertake a period of training and research abroad. Applicants should write to Dr M.M.Black, Honorary Secretary, British Association of Dermatologists, 6 St Andrews Place, Regent's Park, London NW1 4LB for details of regulations. Closing date: 30 January 1987.

*B.A.D. Travelling Fellowships* for 1986 were awarded to Dr J.Ashworth, Dr Ellen F.Lawlor, Dr Tania Phillips, Dr Margaret L.Price, Dr Sarah E.Thomas and Dr W.F.G.Tucker. Applications are invited for these Fellowships for 1987 which will be awarded for a period of not less than 3 weeks. It is anticipated that Fellows would visit various centres either in the U.S.A. and Canada or in Europe, and attend one of the national meetings in dermatology. A grant will be given towards expenses. Application forms from Dr M.M.Black at the above address. Closing date: 30 January 1987.

*The Wycombe Prize 1987.* Applications are invited for this Prize which is offered for an outstanding contribution to dermatology published from a district general hospital or non-teaching centre in the U.K. For regulations, contact Dr M.M.Black at the above address. Closing date: 30 January 1987.

*The Whimster Prize.* Nominations are invited for the above Prize, set up to commemorate the late Dr Ian Whimster, to be awarded on the basis of work (including theses) published in the preceding 3 years in the field of experimental pathology of the skin. Nominations already received will be considered in 1987. For details of regulations, contact Dr M.M.Black at the above address. Closing date: 27 February 1987.

*Stiefel Travelling Grants.* We are pleased to announce that through the auspices of Stiefel Laboratories (U.K.) Ltd, Travelling Grants for 1986 have been awarded to Dr J.I.Harper, Dr S.K.Jones, Dr A.Messenger, Dr R.H.Meyrick Thomas, Dr C.M.E.Rowland Payne and Dr M.H.A.Rustin. Applications are once more requested, and application forms and regulations can be obtained from Dr M.M.Black at the above address.

*Sir Archibald Gray Medal.* This distinguished medal has been awarded to Dr Darrell S.Wilkinson.

*Express Boyd Travelling Scholarships.* Three Travelling Scholarships to the value of £375 each will be available for the purpose of attending the next International Congress of Dermatology in Berlin, 24–29 May 1987. Applicants should submit a curriculum vitae together with a summary, on one side of A4 paper, concerning their reasons for wishing to attend this meeting. Applications to Dr M.M.Black at the above address. Closing date: 30 January 1987.

*B.A.D. Junior Travelling Fellowship.* This new Award was recently approved by the Executive Committee of the British Association of Dermatologists. It is intended for trainees in dermatology who might wish to study abroad for one year only. The value of the Award is £7500. Applicants should submit a curriculum vitae together with a summary of the work to be undertaken, on no more than 3 sides of A4 paper to Dr M.M.Black at the above address. Closing date: 30 January 1987.

*Dermatology Fellowship.* At its last meeting, the Executive Committee of the British Association of Dermatologists approved the setting up of a new Dermatology Fellowship. This Fellowship can be held *only* in the U.K. at an approved centre, and is for one year only. The maximum value of this Award will be £25 000, payable to the supervising department, this sum to include salary and overheads and any technical equipment or support services deemed necessary. Applicants should be at a relatively late stage in their dermatology training (preferably after achieving accreditation) and the Fellowship should be considered as a secondment from an existing Senior Registrar or equivalently graded post in the U.K. It is intended that the Fellowship shall be available for potential applicants wishing to pursue one year's research or gain special expertise in special interest subjects related to dermatology. Applications will be considered by a sub-committee of the British Association of Dermatologists and should be submitted typed on A4 paper and be sufficiently detailed.

Applications should be submitted jointly by a proposed supervisor through the Head of Department, setting out in detail the training scheme or research project planned and the costs, including salary, involved and by the Fellowship candidate enclosing a full curriculum vitae and letter of application. They should be sent to Dr M.M.Black at the above address. Closing date: 27 February 1987.

*Westwood Pharmaceutical Awards* for 1986 have been awarded to the following:

*Research Travelling Scholarship* – Dr Dafydd L.Roberts.

*Travelling Fellowship for Consultants* – Dr Irene Leigh.

*Clinical Photographic Equipment Packages*
Dr W.A.Branford – Kettering & District General Hospital
Dr D.B.Brookes – Furness General Hospital
Dr D.H.Jones – Raigmore Hospital
Dr C.M. Lawrence – North Staffordshire Health Authority
Dr B.N.Statham – Neath General Hospital.

*Award for a highly meritorious paper in the British Journal of Dermatology over the previous year* – Awarded to Dr I.M.Leigh, Dr K.A.Pulford, Dr F.C.S.Ramaekers and Dr E.B.Lane for their paper entitled 'Psoriasis: maintenance of an intact monolayer basal cell differentiation compartment in spite of hyperproliferation' *B.J.D.*, 1985, **113**, 53.

*Two Awards for the best oral presentations at the B.A.D. Annual Meeting*—Dr B.Adriaans, Dr B.Draser and Dr R.Hay: 'The role of anaerobic bacteria in the pathogenesis of tropical (phagedenic) ulcer.' Dr N.H.Cox, Dr D.J.Downey, Dr E.J.Tuyp, Dr S.K.Jones, Dr J.L.Jay, Dr H.Moseley and Professor R.M.MacKie: 'Safety of photochemotherapy—an eight-year follow-up study'.

*Westwood Awards 1987*. Applications for 1987 are invited for: (i) A research travelling scholarship open to senior registrars or consultants to be used by a candidate who is travelling abroad for a specified item of research or training. It is envisaged that the scholarship will be for a period of about 3 months. Value £3000. (ii) Two travelling fellowships for consultants. Value £750 each. (iii) Clinical photographic equipment packages (two or three per year) to non-teaching units. Applicants are requested to give sufficient detail to help the selection committee to come to a fair decision. Applications to Dr M.M.Black at the above address. Closing date: 30 January 1987.

*Bristol Cup 1986*. The Bristol Cup awarded for the best poster at the Annual Meeting of the British Association of Dermatologists was presented to Dr C.R.Lovell and Dr G.Machin for their poster entitled 'Phototoxic plants in Britain'. The runners-up were Dr R.Cerio, Dr M.H.Allen and Dr D.M.MacDonald for their poster entitled 'The detection of extracellular antigens in formaldehyde-fixed paraffin embedded cutaneous tissue'.

## Meeting of the British Association of Dermatologists 1987

The 1987 Meeting of the British Association of Dermatologists, under the Presidency of Dr N.R.Rowell, will be held in Harrogate from Wednesday 24 June to Saturday 27 June. Abstracts are invited for consideration by the Selection Committee. These should be submitted on official abstract forms which are available from Dr M.M.Black, Honorary Secretary, British Association of Dermatologists, 6 St Andrew's Place, Regents Park, London NW1 4LB. Telephone: 01 935 8576. The closing date for receipt of these completed forms is 5 January 1987.

## The 17th ESDR Annual Meeting

The 17th ESDR Annual Meeting will be held in Amsterdam, The Netherlands, from Sunday, 29 March to Wednesday, 1 April 1987. The meeting will be preceded by a postgraduate course—'From Skin Biology to Disease'. This will take place on Sunday, 29 March. Abstracts are available from Dr W.J.Cunliffe, Department of Dermatology, Leeds General Infirmary, Great George Street, Leeds LS1 3EX, U.K. The closing date for abstracts is 15 December 1986. These should also be sent to Dr Cunliffe.

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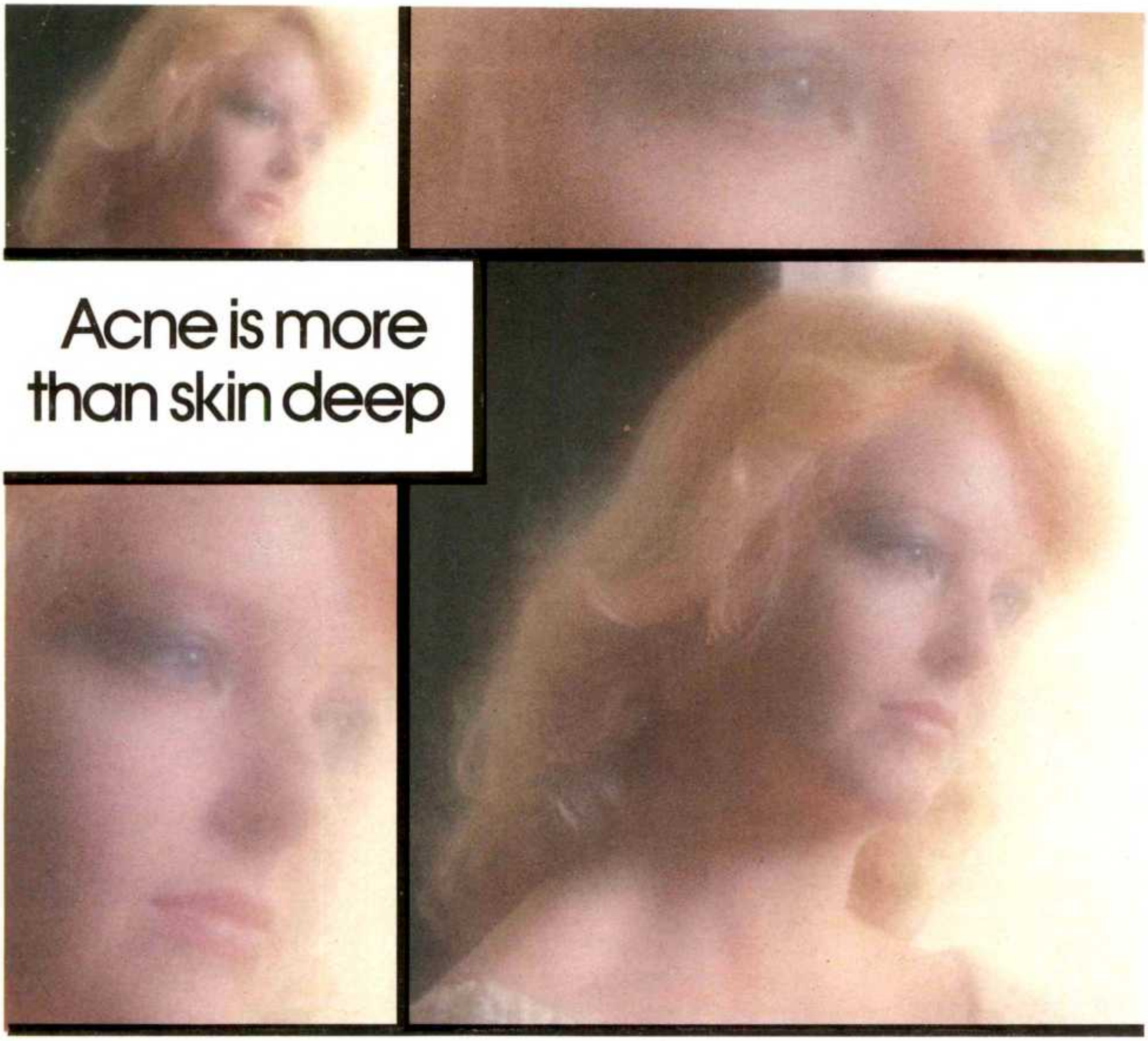

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| | | | | | |
|---|---|---|---|---|---|
| gram(s) | g | minute(s) | min | molar | mol l |
| kilogram(s) | kg | centimetre(s) | cm | millilitre(s) | ml |
| milligram(s) (10$^{-3}$g) | mg | second(s) | s | gravitational acceleration | *g* |
| microgram(s) (10$^{-6}$g) | μg | cubic millimetre(s) | mm$^3$ | micrometre(s) | μm |
| nanogram(s) (10$^{-9}$g) | ng | millimetre(s) | mm | per cent | |
| picogram(s) (10$^{-12}$g) | pg | millicurie(s) | mCi | isotopic mass number placed as | $^{131}$I |
| hours(s) | h | milliequivalent | mmol | | |

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# British Journal of Dermatology, Volume 115, Number 5, November 1986

CONTENTS PAGE

A relative thrombocytosis and elevated mean platelet volume are features of gravitational disease 521
M.J.D.GOODFIELD
Support and stimulation of epidermal cell outgrowth from porcine skin explants by platelet factors 529
PATRICIA A.HEBDA, STEPHANIE P.ALSTADT, W.T.HILEMAN AND W.H.EAGLSTEIN
*In vivo* induction of Ia antigens on rat keratinocytes by $\gamma$-interferon 543
A.SCHEYNIUS, C.JOHANSSON AND P.H.VAN DER MEIDE
The *in vitro* antimicrobial effect of azelaic acid 551
J.P.LEEMING, K.T.HOLLAND AND R.A.BOJAR
Demonstration of a unique viral structure: the molluscum viral colony sac 557
W.B.SHELLEY AND VERONIKA BURMEISTER
Excision of skin tumours without wound closure 563
C.M.LAWRENCE, J.S.COMAISH AND M.G.C.DAHL
No association between malignant melanoma and acne or psoriasis: results from the Western Canada Melanoma Study 573
J.M.ELWOOD, R.P.GALLAGHER AND P.J.STAPLETON
Low-dose etretinate in the maintenance of remission of palmoplantar pustular psoriasis 577
S.I.WHITE, LINDA PUTTICK AND JANET M.MARKS

**Society Proceedings**

Proceedings of the 236th Meeting of the Netherlands Society for Dermatology and Venereology 583

**Case Reports**

Bazex paraneoplastic acrokeratosis: a case report and response to Tigason 595
J.M.WISHART
Acute HTLV III infection associated with exanthema, diagnosed by seroconversion 601
GUNHILD R.LANGE WANTZIN, B.ØRSKOV LINDHARDT, KAARE WEISMANN AND KAY ULRICH
Skin lesions from hypersensitivity to cold during chronic myelomonocytic leukaemia 607
B.DRENO, P.GANDON, B.BUREAU, N.MILPIED AND H.BARRIÈRE
Atypical angiomatosis in polyarteritis nodosa 611
R.F.HORAN, S.R.TAHAN, R.J.MARGOLIS AND H.A.HAYNES
Granulomatous cheilitis and a positive Kveim test 619
S.A.SHEHADE AND I.S.FOULDS
A case of multicentric reticulohistiocytosis: uncommon clinical signs and a report of T-cell marker characteristics 623
C.A.GREEN, D.J.WALKER AND A.J.MALCOLM
Generalized pustular psoriasis precipitated by trazodone in the treatment of depression 629
J.H.BARTH AND H.BAKER
Papular and nodular mucinosis associated with systemic lupus erythematosus 631
F.RONGIOLETTI AND A.REBORA

**Correspondence**

First blast of the trumpet against the monstrous regiment of scientists (with apologies to John Knox) 637
A.W.MCKENZIE
Acro-osteolysis and the nail unit 638
R.K.SCHER
Methotrexate for intractable benign familial chronic pemphigus 640
G.M.FAIRRIS, J.E.WHITE, B.J.LEPPARD AND P.G.GOODWIN
Wallace's Line in serum sickness and Kawasaki disease 640
J.D.BERNHARD, A.R.RHODES AND J.W.MELSKI

**Book Reviews** 641

**News and Notices** 645

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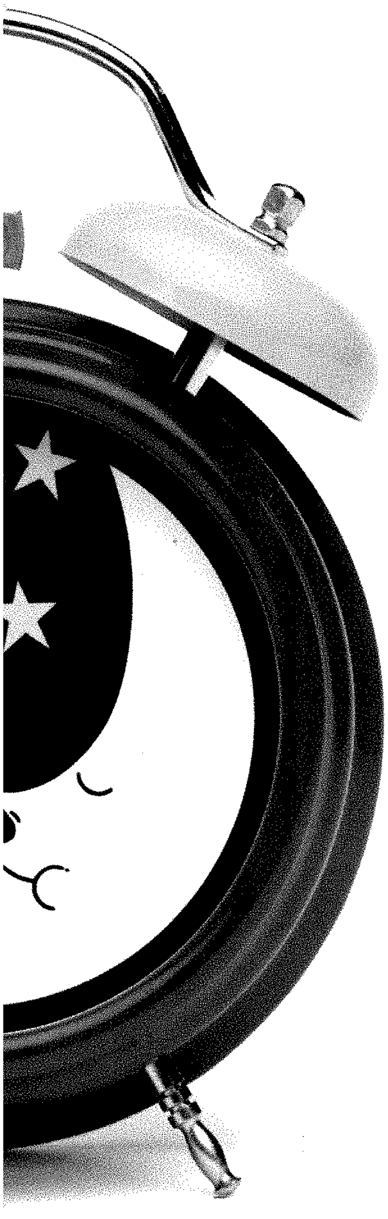

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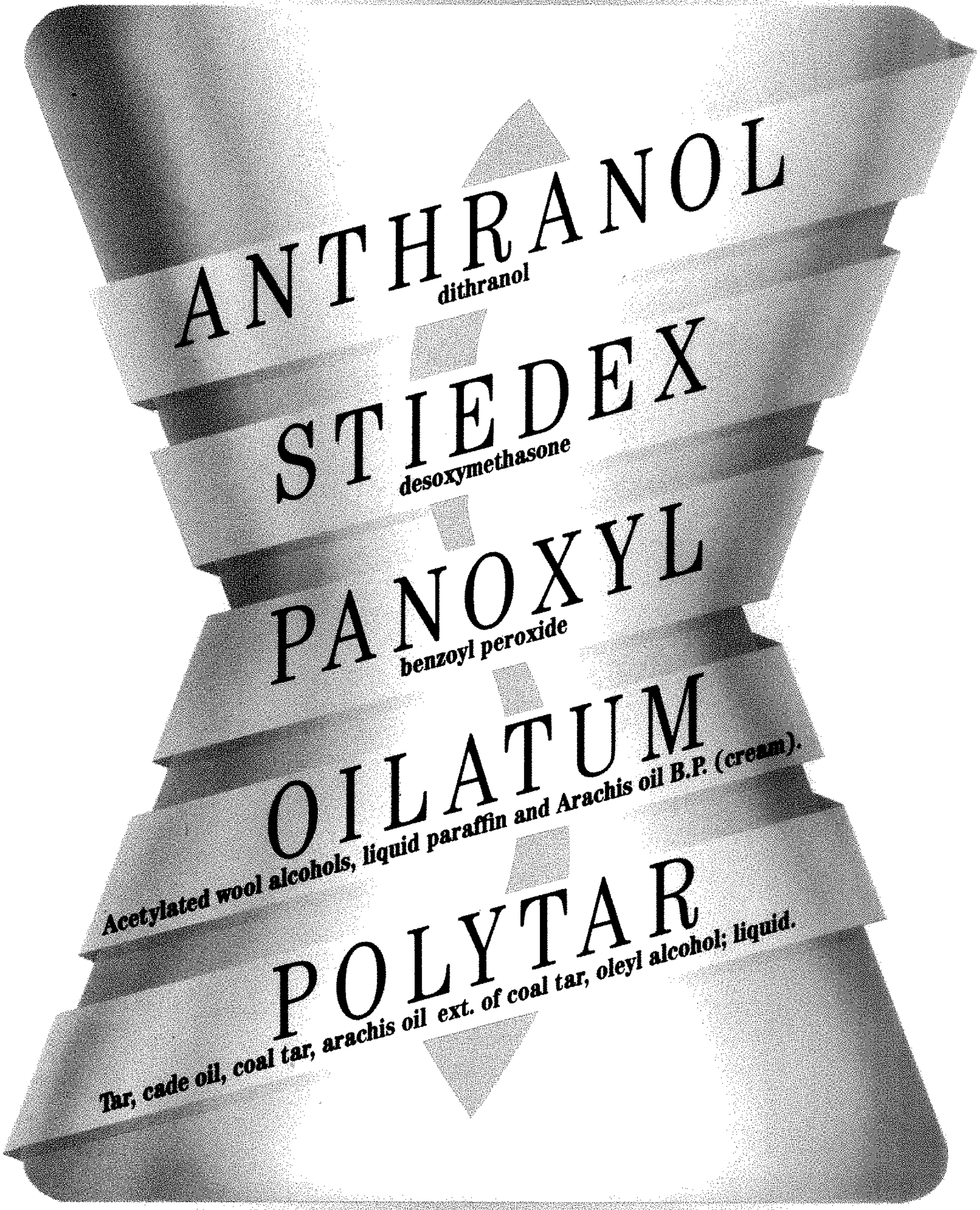
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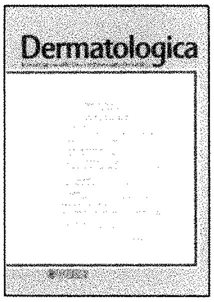

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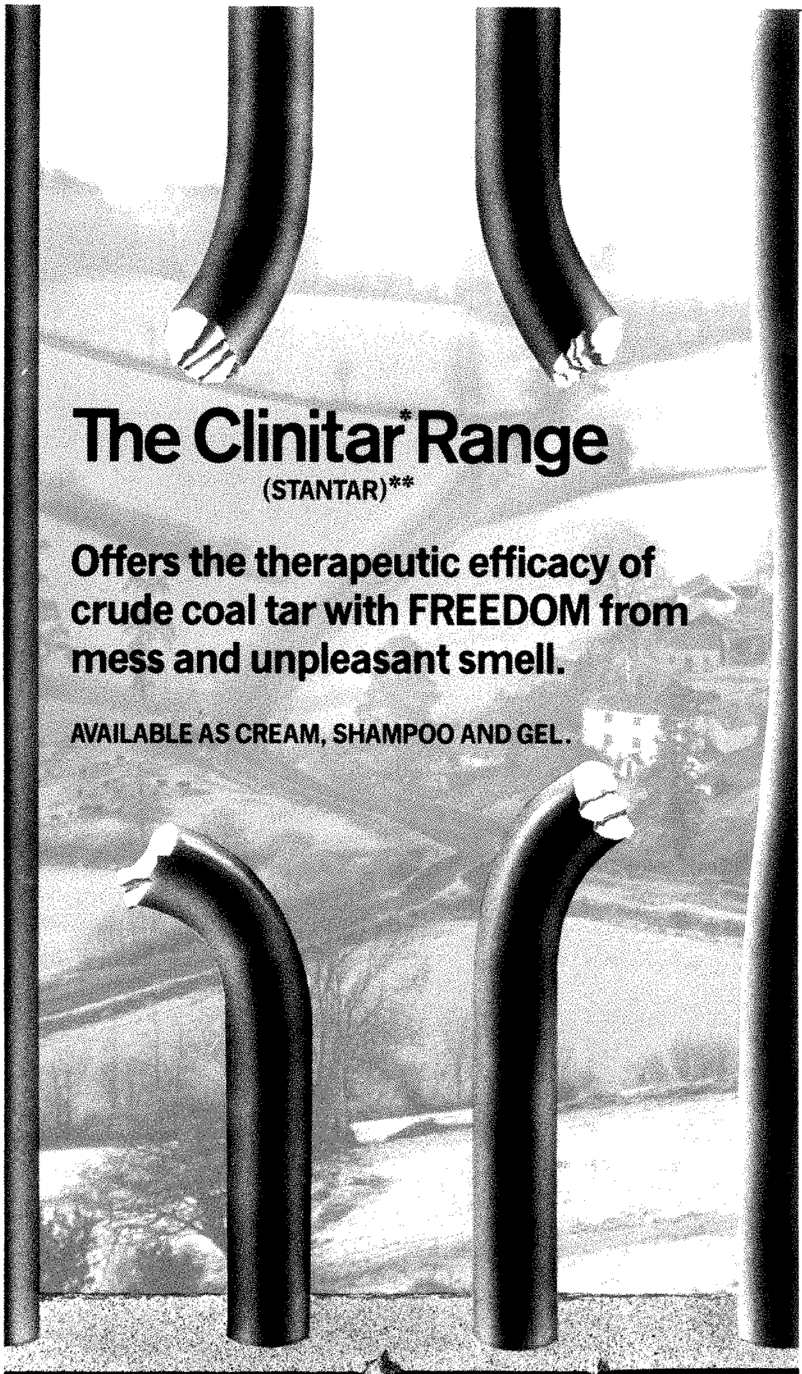
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**USES** For the treatment of sub-acute and chronic psoriasis and eczema
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**CONTRA-INDICATIONS AND WARNINGS** The use of Clinitar cream is not recommended in generalised pustular psoriasis, infections of the skin, or for patients allergic to coal tar. Coal tar can sensitise the skin to U.V. light, leading to sunburn. Clinitar cream should therefore be used cautiously in the sun. Before any irradiation treatment, Clinitar cream should be removed completely
**PHARMACEUTICAL PRECAUTIONS** None
**LEGAL CATEGORY** P
**PACKAGE QUANTITIES** 60g tube
**FURTHER INFORMATION** Clinitar cream contains Stantar, which is an extract of coal tar standardised to have a photosensitising activity corresponding to that of crude coal tar. By using Stantar instead of crude coal tar a product is obtained which is easy to apply, has no unpleasant smell, and is cosmetically inconspicuous
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**BASIC NHS Cost** £2.73

## Clinitar Shampoo

**PRESENTATION** A clear brown shampoo with a slightly greenish fluorescence containing 2% Stantar
**USES** For the treatment of pityriasis simplex capitis (dandruff), seborrhoeic dermatitis of the scalp, and psoriasis of the scalp
**DOSAGE AND ADMINISTRATION** Moisten the hair and rub a little shampoo into the hair and scalp. Rinse and repeat the treatment, this time producing a heavy lather. Leave the lather in the hair for 5 minutes after the second treatment and then rinse carefully. Use 1–3 times a week
**CONTRA-INDICATIONS AND WARNINGS** The use of Clinitar shampoo is not recommended for patients allergic to coal tar. Avoid getting shampoo into the eyes
**PHARMACEUTICAL PRECAUTIONS** None. Store at room temperature
**LEGAL CATEGORY** P
**PACKAGE QUANTITIES** 60g tube
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**BASIC NHS Cost** £2.04

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**DOSAGE AND ADMINISTRATION** Apply to the affected areas once or twice daily
**CONTRA-INDICATIONS AND WARNINGS** The use of Clinitar gel is not recommended in generalised pustular psoriasis, infections of the skin, or for patients allergic to coal tar. Coal tar can sensitise the skin to U.V. light, leading to sunburn. Clinitar gel should therefore be used cautiously in the sun. Before any irradiation treatment Clinitar gel should be removed completely. During the summer it is advisable to apply Clinitar gel only once a day at bedtime followed by a bath the next morning. Simultaneous treatments with a coal tar product and drugs which may provoke photosensitivity may create a potential risk for the patient to acquire unintended sunburn on exposure to the sun or sunlight from an artificial source
**PHARMACEUTICAL PRECAUTIONS** None
**LEGAL CATEGORY** P
**PACKAGE QUANTITIES** 40g tube
**FURTHER INFORMATION** Clinitar gel contains Stantar, which is an extract of coal tar standardised to have a photosensitising activity corresponding to that of crude coal tar. By using Stantar instead of crude coal tar a product is obtained which is easy to apply, has no unpleasant smell, and is cosmetically inconspicuous
**PRODUCT LICENCE NUMBER** 0033/0115
**BASIC NHS Cost** £2.59

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**CONTENTS:**
Future aims.
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Table 3. Products.
Table 4. Photosensitivity.
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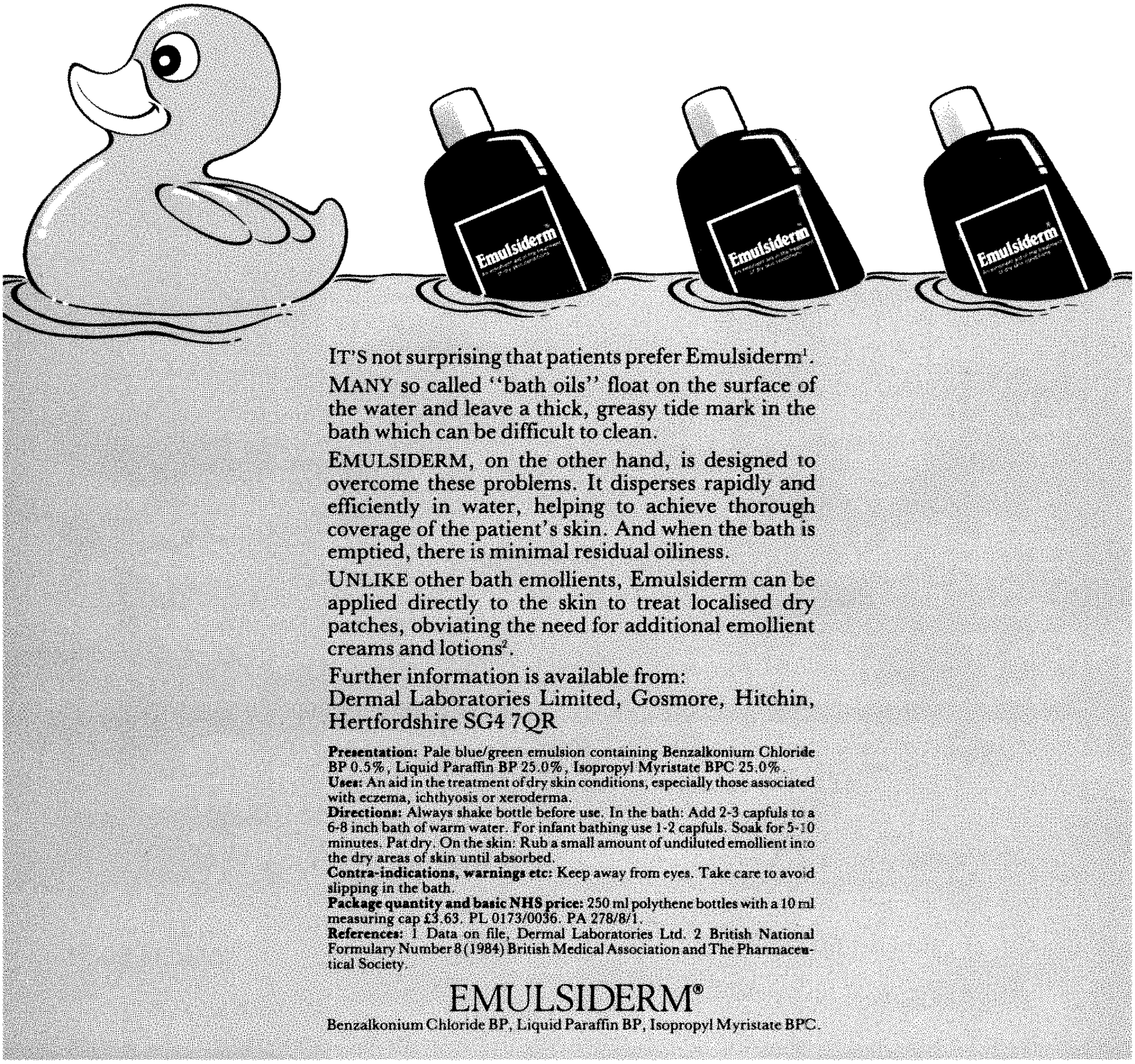

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*British Journal of Dermatology* (1986) **115**, 647–648.

***Comment***

# Nomenclature of non-neoplastic vulval conditions

C.M.RIDLEY

Whittington Hospital, London N19 5NF, U.K.

The International Society for the Study of Vulvar Disease (ISSVD) produced the now well known classification of non-neoplastic vulval conditions in 1976.

| | |
|---|---|
| *Hyperplastic dystrophy* | — with atypia |
| | — without atypia |

(Hypertrophic dystrophy now preferred; ISSVD Congress, 1983).

*Lichen sclerosus*

| | |
|---|---|
| *Mixed dystrophy* | – with atypia |
| | – without atypia |

(Lichen sclerosus with foci of epithelial hyperplasia).

Detailed histological definitions and descriptions were given of each of these conditions. Atypia was subclassified as mild, moderate or severe, with or without dystrophy.

Discussion over the last few years in the ISSVD and the International Society of Gynecological Pathologists (ISGYP) has resulted in a revised classification, accepted by both organizations, for vulval intra-epithelial neoplasia (previously squamous cell carcinoma *in situ*). This is as follows:

| | |
|---|---|
| VIN I | mild dysplasia, |
| VIN II | moderate dysplasia, |
| VIN III | Severe dysplasia or carcinoma *in situ*,<br>Severe dysplasia or carcinoma *in situ* (differentiated type). |

In 1985, the Committee on Nomenclature of Vulvar Dystrophies and Tumors of the ISGYP formulated a new classification, still under review, as follows, for:

*Non-neoplastic epithelial disorders of skin and mucosa*

(a) lichen sclerosus (lichen sclerosus et atrophicus),

(b) squamous hyperplasia, not otherwise specified (NOS),

(c) other dermatoses.

In addition:

(i) Lichen sclerosus has been classified as a dystrophy by the ISSVD.

(ii) Squamous hyperplasia, NOS, is also referred to as hyperplastic dystrophy without atypia by the ISSVD.

(iii) Squamous hyperplasia, NOS, may be associated with varying degrees of inflammation. Some of the lesions with inflammatory changes fulfil the criteria for lichen simplex chronicus (neurodermatitis) and, in such cases, one of these two terms should be used instead of squamous hyperplasia NOS.

(iv) Lichen sclerosus with associated squamous hyperplasia has been classified as mixed dystrophy by the ISSVD. In such cases, it is recommended that both lichen sclerosus and squamous hyperplasia be reported.

(v) In some cases, specific lesions or dermatoses involving the vulva (e.g. psoriasis, lichen planus, candida infection, condyloma acuminatum) may resemble squamous hyperplasia NOS, but should be diagnosed specifically and excluded from this category.

### *Mixed non-neoplastic and neoplastic epithelial disorders*

When lichen sclerosus or squamous hyperplasia is associated with vulvar intraepithelial neoplasia (VIN), both diagnoses should be reported, e.g. lichen sclerosus with VIN I, II or III.

It is felt within the ISSVD that revision of its nomenclature of non-neoplastic conditions may now be profitably attempted. Dermatologists are concerned that the various vulval dermatoses should be clearly defined clinically and histologically, and that the findings of lichenification and lichen simplex at that site should be recognized.

Accordingly, the ISSVD is convening a newly reconstituted sub-committee of gynaecologists, pathologists and dermatologists, which will consider the nomenclature of non-neoplastic vulval conditions and will report at the next conference of the ISSVD in 1987.

One course open to the sub-committee would be to recommend discussion along the lines of the above ISGYP scheme, which appears more flexible and more clinically and dermatologically orientated than the 1976 ISSVD scheme. This would have the advantage of uniting the disciplines. The sub-committee could, moreover, modify or add to the proposed terms, and at this early stage in the new proposals such suggestions could be easily assimilated.

'Squamous hyperplasia', for example, is not a term employed by dermatologists, and it may be that we should aim at the addition of an acceptable synonym. One suggestion already made is that the order would more logically be:

(i) lichen sclerosus (lichen sclerosus et atrophicus),
(ii) other dermatoses,
(iii) squamous hyperplasia, not otherwise specified.

The sub-committee is to be convened and chaired by Dr C.M.Ridley, who would welcome views, discussion and advice on this topic. Correspondence should be addressed directly to her at: The Whittington Hospital, St Mary's Wing, Highgate, London N19 5NF, U.K.

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**Clinical and Laboratory Investigations**

# Spontaneous remission of solar keratoses: the case for conservative management

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## SUMMARY

One thousand and forty people aged 40 years and over, 616 (59·2%) of whom had solar keratoses, were followed for 12 months. Two hundred and twenty-four people (36·4%) had a spontaneous remission of at least one of their solar keratoses. A total of 485 lesions (25·9%) underwent spontaneous remission out of the 1873 lesions that were present at the first examination of these 224 people. There was no significant difference between the number of lesions present at the initial examination in those who had a spontaneous remission compared with those who did not. There was a 21·8% increase in the total number of solar keratoses in the 1040 people studied in the 12-month period, due to new lesions forming at the same time as remissions were occurring. The incidence rate of squamous cell carcinoma occurring in the people with solar keratoses was 0·24% for each solar keratosis present at the original examination.

With a substantial proportion of solar keratoses remitting spontaneously, plus the low rate of malignant transformation and the low potential for metastasis to occur from squamous cell carcinoma arising in a solar keratosis, the rationale of treating all solar keratoses appears questionable.

Solar keratoses are common in fair-skinned people living in sunny climates throughout the world (Marks *et al.*, 1983). Spontaneous remission of solar keratoses has been mentioned briefly in the literature (Sanderson & MacKie, 1979), but the extent to which this occurs has never been documented. A variety of treatments has been recommended, generally on the basis that the lesions are pre-malignant, although the exact rate of transformation is unknown (Montgomery

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& Dorffel, 1932). We present the results of a population-based prospective study of spontaneous remission in a fixed population over a 12-month period.

## METHODS

The spontaneous remission of solar keratoses was investigated as part of a longitudinal study, commenced in 1982, on the incidence of non-melanoma skin cancer in an Australian population. The normal population of Maryborough, a rural city in North Central Victoria, has been studied annually on a voluntary presentation basis. In this survey, 2835 of the eligible population of 3243 people have been studied to date.

The survey team consisted of a consultant dermatologist, a dermatology registrar in training, and a B.Med.Sci. student doing an honours year in dermatology. Prior to the commencement of the study, this team was tested for its ability to make clinical diagnoses of solar keratoses. Thirty-six patients were chosen who had what all the members of the team felt were clinically typical solar keratoses. A biopsy was performed on the lesions and the diagnosis was confirmed in 34 (94%) of the 36 patients. The other two lesions were reported as a seborrhoeic keratosis in one, and no abnormality apart from severe elastosis seen in the other.

All people examined were aged 40 years and older, and the examination included the light exposed areas of the face, head and neck, hands and forearms. The people reported on here were seen in 1983, and the sites of all solar keratoses diagnosed were recorded on a personal record chart. Patients were informed of the nature of the lesions seen, and were told that treatment was not necessary unless some obvious change occurred prior to their next examination. The following year they were re-examined and again all solar keratoses were recorded on the same chart with any remission or new solar keratosis being recorded. Details of any treatment in the intervening 12-month period were also obtained. Diagnoses were made on clinical assessment, except when there was some doubt, in which case a biopsy was performed.

Demographic data were obtained from each person, including age, sex, country of birth, number of years of residence in Australia, recreational activities, past history of skin cancer, and response to sunlight, including burning or tanning ability. In addition, certain physical characteristics were recorded, including eye colour, hair colour and skin complexion.

All data were punch coded and verified before being entered onto a Burroughs B6700 computer at Monash Univesity. Statistical analysis of the results was completed with the Statistical Package for the Social Sciences (Nie *et al.*, 1975; SPSS$^x$, 1983).

## RESULTS

One thousand and forty people were seen in 1983, 446 men (42·9%) and 594 women (57·1%), mean age 58·8 years (range 40–99 years). Four hundred and twenty four (40·8%) had no solar keratosis at the original visit and 616 (59·2%) had at least one solar keratosis. The mean age of those people with solar keratoses (62·2 ± 10·5 years) was significantly higher than those without solar keratoses (54·0 ± 10·0 years) ($P < \cdot 001$; Student's *t*-test). The subgroups showing numbers of people who had no change, remissions, new lesions and/or treatment of solar keratoses in 1983–84 are shown in Figure 1 and Table 1. Three of the 616 people with solar keratoses had a total of 11 lesions treated by the local medical practitioner in 1983 and were excluded from analysis of the data.

Of the 613 untreated people who had solar keratoses in 1983, 224 (36·4%) had at least one spontaneous remission. A total of 485 lesions (25·9%) remitted out of the 1873 lesions that were

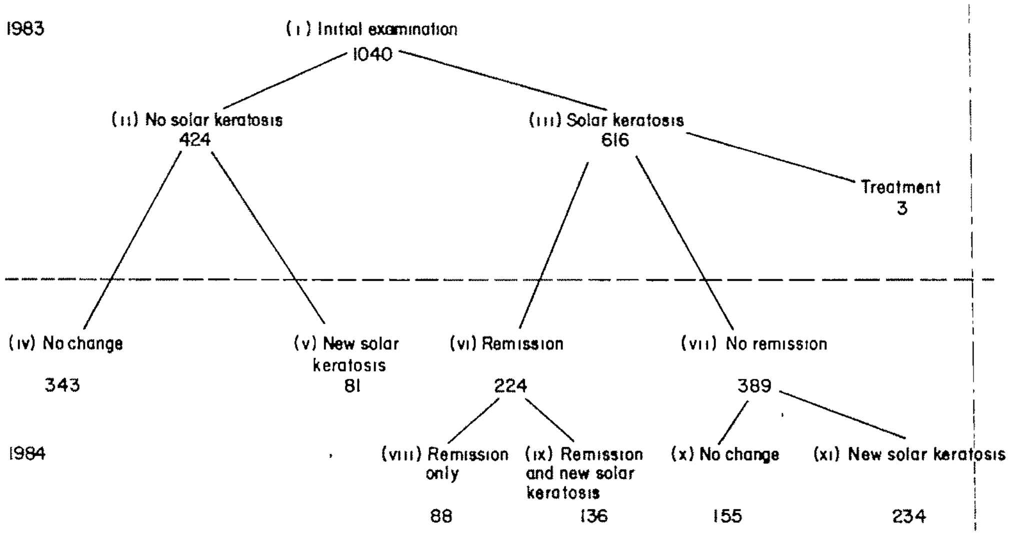

FIGURE 1. Diagram showing numbers of people who had no change, remissions, new lesions and treatment of solar keratoses in 1983–84.

TABLE 1. Age, sex, remissions and new lesions in the subgroups of the people examined in 1983–84 (see Fig. 1)

| Subgroup (Fig. 1) Number of people | Age (years) mean ± SD | Men (%) | Women (%) | Number of lesions in 1983 (number/person) | Number of remissions (number/person) | Number of new lesions (number/person) | Total number of lesions in 1984 (number/person) |
|---|---|---|---|---|---|---|---|
| (i) 1040 | 58·8 ± 11·1 | 446<br>(42·9) | 594<br>(57·1) | 4746<br>(4·6) | 485<br>(0·5) | 1535<br>(1·5) | 5796<br>(5·6) |
| (ii) 424 | 54·0 ± 10·0 | 146<br>(34·4) | 278<br>(65·6) | — | — | 175<br>(0·4) | 175<br>(0·4) |
| (iii) 613 (minus three treated) | 62·2 ± 10·5 | 300<br>(48·7) | 316<br>(51·3) | 4717<br>(7·7) | 485<br>(0·8) | 1360<br>(2·2) | 5592<br>(9·1) |
| (iv) 343 | 53·5 ± 10·1 | 112<br>(32·7) | 231<br>(67·3) | — | — | — | — |
| (v) 81 | 56·0 ± 9·2 | 34<br>(42) | 47<br>(58) | — | — | 175<br>(2·2) | 175<br>(2·2) |
| (vi) 224 | 62·4 ± 10·5 | 112<br>(50) | 112<br>(50) | 1873<br>(8·4) | 485<br>(2·2) | 423<br>(1·9) | 1811<br>(8·1) |
| (vii) 389 | 61·9 ± 10·6 | 286<br>(47·8) | 203<br>(52·2) | 2844<br>(7·3) | — | 937<br>(2·4) | 3781<br>(7·7) |
| (viii) 88 | 61·6 ± 10·5 | 44<br>(50) | 44<br>(50) | 464<br>(5·2) | 190<br>(2·2) | — | 274<br>(3·1) |
| (ix) 136 | 62·9 ± 8·9 | 68<br>(50) | 68<br>(50) | 1409<br>(10·4) | 295<br>(2·2) | 423<br>(3·1) | 1537<br>(11·3) |
| (x) 155 | 60·6 ± 10·9 | 67<br>(43·2) | 88<br>(56·8) | 705<br>(4·5) | — | — | 705<br>(4·5) |
| (xi) 234 | 62·9 ± 10·2 | 119<br>(50·9) | 115<br>(49·1) | 2139<br>(9·1) | — | 937<br>(4·0) | 3076<br>(13·1) |

present on the first examination in those 224 people. There were originally 4746 solar keratoses in the 616 people seen in 1983, an average of 7·7 solar keratoses per person. There was no significant difference between the number of lesions in people who had a remission and those who did not ($P > 0·05$; Mann-Whitney U test). Four hundred and fifty-eight (44%) of the 1040 persons examined in 1983 developed at least one new solar keratosis in the 12-month period covered by the present study, giving an overall increase in the total number of solar keratoses of 22·4%. The proportions of people who developed new lesions did not differ significantly between those who had a spontaneous remission and those who did not ($P > 0·05$; $\chi^2$ test).

To determine whether any of the factors measured isolated those people who had a remission, a logit model was used. This model included the explanatory variables: age group, sex, response to sunlight, and present occupation, or past if retired. This model generated the odds on remission as the response function, which could be explained by some explanatory variables (or interactions). The odds on remission were defined as the ratio of the probability of lesions remitting to the probability of non-remission over the 12-month period. Because only 9·7% of the females (who represented 50·0% of the total study population) were outdoor workers, the process of modelling was focused on males only. The backward-deletion method (i.e. starting with the fully saturated model) was applied. The final model was selected on the basis that (a) the parameter estimates of higher order explanatory variables, i.e. the contribution of two- or three-way interaction terms, were significant at $\alpha = 0·05$, (b) all the adjusted residuals fell within a normal range of $-1·96$ to $1·96$, or (c) the likelihood ratio Chi-square statistic was not significant (i.e. a goodness of fit test). The estimated relative odds were then obtained based upon the expected count from this model (Table 2).

There was a significant chance of remission occuring in the age groups 40–49 years and 70–79 years, if the person studied had worked mainly out-of-doors in the past or was doing so at the time of the first examination. For the age groups 50–69 years, there was little difference in the chance of a remission occuring between the different occupations and the different responses to sunlight. Although the relative odds for remission inclined towards those who 'never burn, just tan' in each age and occupation group, the differences did not reach statistical significance.

In the 12-month period between examinations, 12 people developed a total of 14 squamous cell carcinomas. Of the 613 people with solar keratoses in 1983 who did not have any treatment in the 12-month period, 10 people developed a total of 11 squamous cell carcinomas. This means that for every squamous cell carcinoma which occurred in the 12-month period, there were 429

TABLE 2. Relative odds for remission of solar keratosis in males, based on the logit model analysis. The relative odds are expressed as a comparison of each occupation and response to sunlight group, to the group with the lowest tanning ability and mainly indoor occupation in each age group

| | Age group | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 40–49 years | | | 50–59 years | | | 60–69 years | | | 70–79 years | | |
| Response to sunlight* • | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Occupation: | | | | | | | | | | | | |
| mainly outdoor | 16·77 | 22·86 | 26 95 | 0·94 | 1·29 | 1·59 | 1·01 | 1·38 | 1·63 | 4·80 | 6·57 | 7·82 |
| mainly indoor | 1 00 | 1·12 | 1·62 | 1·0 | 1·17 | 2·50 | 1 00 | 1 18 | 6·55 | 1·00 | 1·15 | 2 47 |

* 1 = Always burn, never tan; 2 = burn first and then tan; 3 = never burn, just tan.

solar keratoses, i.e. an annual incidence rate for squamous cell carcinoma of 0·24% for every lesion of solar keratosis present at the first interview.

## DISCUSSION

The majority of diagnoses made in this study were clinical, except where there was some doubt, in which case a biopsy was performed. This may have introduced some bias. However, the team had been tested prior to the study and the two senior members were the same in both years. Testing the remission rates in people seen by the different members of the team revealed no significant differences between any of them. Although it would be ideal to have histological proof of the diagnosis before and after remission, any remission which followed a biopsy could not be said to be spontaneous.

Solar keratoses, in contrast to the average seborrhoeic keratosis, are inflamed and characterized by erythema associated with the scaling. Histologically, the inflammatory response may be fairly dense, comprised mainly of lymphocytes, but may also contain plasma cells (Pinkus, Jallad & Mehregan, 1963). These findings suggest that the body is responding to the presence of the abnormal lesions and may be trying to mount an immune response to them. Presumably when the response is sufficient, the lesions will regress and that may explain our observation of spontaneous remission. Lichenoid solar keratoses have been reported in the past and have been suggested to be an example of spontaneous immunological regression in solar keratoses (Tan & Marks, 1982).

It has also been reported that solar keratoses may remit if sunlight exposure is reduced (Sanderson & MacKie, 1979). Our analysis of those in whom remission occured fits in with the concept that a reduction in sunlight exposure leads to an increased chance of remission. In the elderly age group of 70–79 years, there was likely to be a reduction of exposure from their previous outdoor occupation upon retirement. In the age group of 40–49 years, it is probable that they reduced their sunlight exposure following advice that they had a solar keratosis at their first visit. The age groups of 50–69 years may not have altered their behaviour much because of less regard for these lesions which are almost universal in their peers. In all the categories, those people who just tanned in response to sunlight were likely to have more sunlight exposure than those who burn easily. Therefore, they would be in more of a position to reduce substantially their sunlight exposure than the people sensitive to sunlight who would already be protecting themselves if possible.

Conversely, patients often presented with lesions whose onset was noticed during the summer, suggesting that the lesions may become more active following sunlight exposure. Recent advances in our knowledge of the role of Langerhans cells in immune surveillance in the epidermis and the effect of sunlight on these cells could partly explain the seasonal variation (Kripke & Morison, 1985). For example, increased sunlight exposure in summer may reduce the immunological response to the dysplastic lesions and allow them to become more apparent.

At the same time as remissions were occuring in this study, new lesions appeared in many of the people we observed. In fact, there was an overall increase in the absolute number of keratoses counted in the 1040 people observed over the 12-month period. This suggests that solar keratoses are in a state of continual flux, i.e. coming and going over a period of time. On the data from this study, one would expect that as individuals age, the number of lesions acquired would also gradually increase. This has been borne out by previous studies in which there is a regular increase in the prevalence of solar keratoses with age (Marks *et al.*, 1983). Because of the tendency to spontaneous remission and occurence of new lesions, care is required in assessing studies purporting to show a response to treatment over a period of time (Moriarty *et al.*, 1982).

Solar keratoses are common in fair-skinned people. A recent study in Australia reported a prevalence of these lesions in more than 50% of persons aged 40 years and over (Marks *et al.*, 1983). A variety of treatments are used, including cryotherapy, curettage and diathermy, excision, topical cytotoxics, and in the past, radiotherapy (Sanderson & MacKie, 1979). Around 12% of patients attending dermatology practices in Australia do so for treatment of solar keratoses (Nurse, 1975). The reasons given for treatment are generally either malignant potential, irritation, or cosmetic. Solar keratoses are said to be pre-malignant lesions progressing to squamous cell carcinoma, although the rate of malignant transformation is unknown and estimates have varied up to a maximum of 20% (Montgomery & Dorffel, 1932). The risk appears to be high in renal transplant patients who are immuno-suppressed (Marshall, 1974). In our study, the annual incidence rate of squamous cell carcinoma was 0·24% for each solar keratosis present at the initial examination. These preliminary results suggest a lower transformation rate than has been reported previously. Squamous cell carcinoma arising in a solar keratosis is considered to have a very low risk of metastasis. A recent study in Australia reported a metastasis rate of 1·6% for squamous cell carcinoma arising in light-exposed areas (Nixon, Dorevitch & Marks, 1986). Given that a substantial proportion of solar keratoses remit spontaneously, that the risk of malignant transformation is low, and that when it does occur the risk of metastatic spread is also low, should we be treating the lesions? Solar keratoses are more frequent in those patients with skin cancer than those without and vice versa (Marks *et al.*, 1983). These lesions may merely be a marker indicating that a person has the right skin type and has had sufficient sunlight exposure to develop skin cancer, rather than being a problem in themselves.

There is no doubt that thickening in a solar keratosis requires investigation to exclude malignant transformation, and adequate treatment if this has occurred. However, the cost of treating all the solar keratoses which are occuring may be considerable for both the patient and the community. The benefit at this stage appears questionable, and advice about sunlight exposure may be more appropriate.

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# Pulmonary function in patients on long-term, low-dose methotrexate

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## SUMMARY

We have made a detailed study of pulmonary function in patients receiving long-term, low-dose methotrexate for psoriasis. We were unable to detect any changes indicative of early pulmonary damage.

In high dosage, methotrexate can produce an acute interstitial pneumonitis, which may occasionally progress to pulmonary fibrosis (Sostman, *et al.*, 1976). The low doses of methotrexate used in the treatment of psoriasis very rarely cause such complications. We have recently encountered two patients, described below, who developed pulmonary reactions to low-dose methotrexate therapy.

It is known that in patients receiving other cytotoxic drugs such as bleomycin, abnormalities in pulmonary function can be detected before the patient becomes symptomatic (Luursema *et al.*, 1983). To date, there have been no studies examining pulmonary function in patients receiving long-term, low-dose methotrexate treatment for psoriasis. We have therefore studied a small group of such patients, to determine whether any abnormalities of pulmonary function could be detected.

## CASE REPORTS

### *Case 1*

This 34-year-old lady had been receiving methotrexate 25 mg weekly for 11 years. Apart from her psoriasis, she was quite healthy. There was no history of occupational exposure, and she did not keep any birds or other pets. She presented with a 2-month history of rapidly progressive exertional dyspnoea, and a productive cough. Examination revealed no finger clubbing or cyanosis, and bilateral coarse crackles at the lung bases. Her blood count showed an eosinophilia of 2000/ml. Repeated sputum cultures were negative, and mycoplasma and legionella titres were not raised. Aspergillus and avian precipitins were negative. Aspergillus skin prick tests were

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Cases 1 and 2 are presented in detail elsewhere (Phillips, 1986).

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TABLE 1 Lung function tests in Cases 1 and 2

| | | | VC (l) | FEV$_1$ (l) | FEV$_1$/VC (%) | TLC (l) | RV (l) | TLCO (mmol/min/KPa) | KCO (mmol/min/KPa) | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| *Case 1* | June 1984 | Observed | 2·46 | 2·15 | 87 | 4·57 | 1·65 | 3·61 | 0·97 | Marked reduction in gas transfer |
| | | Expected | 3·38 | 2·83 | 84 | 4·90 | 1·51 | 8·64 | 1·83 | Lung volumes normal |
| | | % | 73 | 76 | | 93 | 109 | 42 | 53 | |
| | September 1984 | Observed | 3·48 | 2·99 | 86 | 5·35 | 3·26 | 7·59 | 1·70 | Lung function has returned to normal |
| | | Expected | 3·34 | 2·8 | 84 | 4·90 | 2·63 | 8·60 | 1·83 | |
| | | % | 104 | 107 | | 109 | 124 | 88 | 93 | |
| *Case 2* | January 1984 | Observed | 1·56 | 1·11 | 71 | 2·23 | 0·61 | 3·80 | 0·62 | There is a restrictive defect |
| | | Expected | 1·7 | 1·33 | 78 | 2·43 | 0·76 | 10·06 | 1·44 | with loss of lung volume and |
| | | % | 92 | 83 | 91 | 92 | 80 | 38 | 43 | low gas transfer |
| | March 1985 | Observed | 1·46 | 1·06 | 73 | 2·23 | 0·76 | 3·30 | 0·56 | |
| | | Expected | 1·70 | 1·33 | 78 | 2·43 | 0·76 | 10·06 | 1·44 | |
| | | % | 85 | 79 | 93 | 92 | 100 | 33 | 39 | |

negative. Antinuclear factor, autoantibody screens and rheumatoid factor were negative, and no evidence of parasites could be found. Her chest X-ray was normal. Lung function tests showed a marked reduction in gas transfer (observed TLCO 3·61 mmol/min/kPa, predicted 8·64). She had received a total dosage of 9·8 g of methotrexate, which was stopped. Her symptoms improved gradually, and 3 months later her pulmonary function had returned completely to normal (Table 1).

### *Case 2*

This 59-year-old man had received a total dosage of 9·1 g of methotrexate over 7 years for his psoriasis. He presented with gradually worsening exertional dyspnoea. There was no previous history of bronchitis, asthma, heart disease, occupational exposure or exposure to external allergens. He had smoked 15 cigarettes a day for 10 years. On examination there was no finger clubbing or cyanosis, and his chest was clinically clear. His chest X-ray was normal, as well as his blood count and differential white cell count. Autoantibody screens, rheumatoid factor, Aspergillus and avian precipitins were negative. His lung function tests showed low gas transfer (observed TLCO 3·80, mmol/min/kPa, expected 10·06) and hypoxia ($pO_2$ 67 mm Hg). Six months after stopping methotrexate his lung function has shown no improvement (Table 1).

## METHODS

A group of 10 psoriatic patients was selected, who had received long-term, low-dose methotrexate. They had not received any other systemic anti-psoriatic treatment, but one patient was receiving gold for psoriatic arthropathy. This group was compared with 10 psoriatic patients, matched for age, sex and smoking history, who had never received any systemic therapy. Respiratory symptoms were assessed using the MRC Questionnaire on Respiratory Symptoms (1976). Physical examination, chest X-ray, electrocardiograms and pulmonary function tests were performed in all patients. Arterial blood gases were measured in 18 patients.

Forced expiratory volume in one second ($FEV_1$) and forced vital capacity (FVC) were determined using a Vitalograph® spirometer, and peak flow rate (PEFR) with a Wright's peak flow meter. Lung volumes were measured by closed circuit helium dilution (PK Morgan TLC® test). The transfer factor of the lung for carbon monoxide (TLCO) as well as the transfer coefficient (KCO) were measured by the single breath technique (PK Morgan Transfer Test Model C®). Arterial blood gases were collected anaerobically by radial artery puncture using 1% lignocaine chloride local anaesthesia, and analysed within 5 min in a Radiometer ABL1 Blood Gas Analyser®. At the time of the study, venous blood was collected for haemoglobin measurement. The transfer factor and transfer factor coefficient were corrected to a standard haemoglobin concentration of 14·6 g/dl (Cotes, 1979).

Results in the two groups were compared using Student's *t*-test.

## RESULTS

In the methotrexate group, patients had received methotrexate for between 1·5 and 14 years. The total dosage received ranged from 1·34 to 6·9 g, mean 3·4 g. The duration of psoriasis was greater in the methotrexate group, reflecting the more severe disease in these patients (Table 2).

No patient had any significant symptoms or history of cardiorespiratory disease. Five subjects in each group were current smokers, and were matched for numbers of cigarettes smoked daily.

TABLE 2. Details of patients studied

| | Methotrexate group | Control group |
|---|---|---|
| Sex | 6 F, 4 M | 6 F, 4 M |
| Age (years) | 48·1 (33–69) | 46·8 (29–74) |
| Weight (kg) | 63·2 (55–83) | 70·0 (57–92) |
| Height (m) | 1·65 (1·51–1·77) | 1·65 (1·33–1·76) |
| Duration of psoriasis (years) | 25·7 (4–47) | 8·6 (2 – 30) |
| Total dose of methotrexate (g) | 3·4 (1·4–6·9) | |
| Duration of treatment (years) | 6·3 (1·5–14) | |

* Values are mean and range throughout.

TABLE 3. Lung function tests on patients treated with methotrexate and on controls

| Test | Methotrexate | Control group |
|---|---|---|
| $FEV_1$ (l) | 3·0 ± 0·6 (110 ± 13) | 3·2 ± 0·9 (110 ± 22) |
| FVC (l) | 4·0 ± 1·0 (111 ± 13) | 3·9 ± 0·9 (110 ± 16) |
| $FEV_1$/FVC (%) | 77 ± 8 | 82 ± 7 |
| PEFR (l/min) | 465 ± 65 (91 ± 7) | 462 ± 104 (90 ± 9) |
| TLC (l) | 6·1 ± 1·5 (119 ± 35) | 5·5 ± 1·0 (104 ± 12) |
| RV (l) | 2·3 ± 1·0 (120 ± 33) | 1·7 ± 0·3 (93 ± 13) |
| TLCO (mmol/kPa/min) | 8·4 ± 0·8 (100 ± 12) | 8·9 ± 1·0 (106 ± 14) |
| KCO* (mmol/kPa/min/l) | 1·6 ± 0·3 (95 ± 12) | 1·8 ± 0·2 (105 ± 19) |
| $PaO_2$ (kPa) | 12·4 ± 0·8 | 12·4 ± 0·4 |

Results are mean ± SD.

Values in parenthesis are the percentages of predicted normal values (see text).

* Corrected to standard haemoglobin concentration of 14·6 g/dl.

$FEV_1$ = forced expiratory volume in 1 second; FVC = forced vital capacity; PEFR = peak expiratory flow; TLC = total lung capacity; RV = residual volume; TLCO = transfer factor of the lung for carbon monoxide; KCO = transfer coefficient; $PaO_2$ = arterial oxygen partial pressure.

Two subjects in the control group and three in the methotrexate group were ex-smokers. Physical examination, chest X-ray, and electrocardiography were normal in all the subjects.

In the methotrexate group, two patients showed evidence of mild irreversible airflow obstruction with gas trapping and an increase in residual volume. These patients were either current or ex-smokers. The remaining patients in this group had normal pulmonary function tests. In the control group, two patients had evidence of mild airflow obstruction which was reversed by salbutamol; in the remaining subjects, pulmonary function tests were within normal limits.

There were no statistically significant differences in PEFR, spirometric and lung volume results, or indices of gas transfer, between the two groups. Arterial blood gas tensions were also normal in all the subjects in which they were measured, and there were no statistically significant differences between the two groups (Table 3).

## DISCUSSION

Pulmonary damage due to methotrexate was first described in 1969, in patients receiving high doses for the treatment of acute lymphoblastic leukaemia (Acute Leukaemia Group, 1969). Since then, methotrexate-induced pneumonitis has become well recognized (Sostman *et al.*, 1976). Patients present with cough, fever, dyspnoea and cyanosis, occurring 10 days to 6 months after commencing high-dose methotrexate treatment. Bilateral basal crackles are found in the chest, and there is often a peripheral eosinophilia. The chest X-ray may demonstrate bilateral pulmonary infiltrates, while lung function tests reveal low gas transfer and hypoxia. Providing the drug is discontinued, the pneumonitis is often reversible. Occasionally, however, progression to irreversible pulmonary fibrosis can occur (Nesbit *et al.*, 1976). Pulmonary damage most often follows high dosage schedules of methotrexate, but total doses as small as 40 mg have been reported to produce pneumonitis (Everts, Westcott & Bragg, 1973).

Patients taking methotrexate for psoriasis are also at risk, although the incidence of pulmonary complications in this group appears to be low. Sostman *et al.*, (1976) described two psoriatic patients who developed acute pneumonitis after taking 100 mg and 375 mg of methotrexate respectively, given in weekly doses of 10–20 mg. In both patients, the pneumonitis resolved within days of stopping the drug. To date, there have been only two case reports of pulmonary fibrosis following long-term, low-dose methotrexate for psoriasis. Kaplan and Waite (1978) reported a patient who developed exertional dyspnoea, diffuse interstitial shadowing on chest radiography, and impaired gas transfer on pulmonary function testing, after receiving approximately 20 g of methotrexate over 18 years. Lewis and Walter (1979) described a similar patient who developed breathlessness and diffuse interstitial shadowing on chest radiography after receiving 24 g of methotrexate over 17 years. In both cases, pulmonary impairment persisted despite discontinuing methotrexate.

In this report, we have described two further patients who developed pulmonary complications after long-term, low-dose methotrexate treatment. These patients prompted us to examine in some detail the pulmonary function in psoriatic patients receiving methotrexate. Lung function studies in patients receiving other cytotoxic agents such as bleomycin (Luursema *et al.*, 1983) have been able to detect early pulmonary damage before overt pneumonitis appeared, enabling more serious pulmonary reactions to be averted. The present study is the first in which detailed pulmonary function tests have been performed in asymptomatic patients on long-term methotrexate treatment. We were unable to detect any changes indicative of early pulmonary damage; pulmonary function tests, including measurements of gas transfer, were within normal limits in every case. In the group as a whole, the results did not differ significantly from a closely-matched group of psoriatic patients who had never received methotrexate.

At present, the incidence of pulmonary complications in patients with psoriasis receiving long-term methotrexate treatment is unknown. Whether the total dosage received is important remains unclear. Both our patients who developed pulmonary complications had received large total doses of methotrexate. Those patients in the group we studied had received a lower dosage, and had no evidence of pulmonary impairment. However, some studies suggest that total dosage of methotrexate and duration of treatment bear no relationship to pulmonary toxicity. These issues can only effectively be answered by long-term prospective studies.

This report should serve to remind clinicians that serious pulmonary complications can occur in patients receiving long-term methotrexate therapy, and should be considered in any patient on methotrexate who develops respiratory symptoms. Pulmonary function may be restored to normal if treatment is discontinued promptly. Reassuringly, we have found no evidence of occult pulmonary damage in asymptomatic patients taking the drug on a long-term basis for psoriasis.

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# The effect of altering area of application and dose per unit area on sensitization by DNCB

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## SUMMARY

We have investigated the contributions of dose per unit area and area of application to the degree of sensitivity induced by dinitrochlorobenzene (DNCB). Subjects sensitized with 35·4 μg/cm$^2$ showed equal sensitization whether the total dose was 250 μg applied to 7·1 cm$^2$ or 62·5 μg applied to 1·8 cm$^2$. Three groups of subjects were sensitized with 16·4 μg/cm$^2$ applied as 56 μg on 3·5 cm$^2$, 116 μg on 7·1 cm$^2$ or 232 μg on 14·2 cm$^2$. Although the total dose changed by a factor of four, the proportions of subjects sensitized were comparable (66, 50 and 55% respectively), and their degrees of reactivity were similar. This contrasts with previous findings that when the area of sensitization was fixed and the concentration was increased over a similar range, highly significant increases in the proportion of sensitized subjects and degree of reactivity occurred.

The factors which determine whether exposure to an antigen will result in sensitization are largely unknown. One of the main factors is the antigenic potency of the substance, but there are many other components which are likely to contribute, including the quantity and concentration of the antigen and the site and the number of exposures. We have shown previously that sensitization by a potent antigen, dinitrochlorobenzene (DNCB), follows predictable log-dose response relationships (Friedmann *et al.*, 1983). In that study, a standard procedure was used, in which different sensitizing doses of DNCB (ranging from 62·5 μg up to 1000 μg) were applied to a circular area 3 cm in diameter. One month later, responsiveness was determined by measurement of responses to a graded series of challenge doses (including 3·125, 6·25, 12·5 and sometimes 25 μg) applied simultaneously on standard 1 cm diameter patch test pads (Al-test). We observed that when applied to subjects who were not sensitized, the challenge doses could act as an effective sensitizing stimulus, with greater potency even than 125 μg applied to the 3 cm diameter area. Since the challenge doses totalled only 46·9 μg, it was apparent that the smaller

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TABLE 1. Site and sensitizing dose of subjects studied

| Number of subjects | Number sensitized | Application site: Diameter cm | Application site: Area cm² | Sensitization dose: Total μg | Sensitization dose: Concentration μg/cm² |
|---|---|---|---|---|---|
| 24 | 2 | 3·0 | 7·1 | 62·5 | 8·8 |
| 40 | 25 | 3·0 | 7·1 | 125 | 17·7 |
| 30 | 25 | 3·0 | 7·1 | 250 | 35·4 |
| 30 | 30 | 3·0 | 7·1 | 500 | 71·0 |
| 8 | 8 | 3·0 | 7·1 | 1000 | 142·0 |
| 7 | 6 | 1·5 | 1·8 | 62·5 | 35·4 |
| 22 | 12 | 2·1 | 3·5 | 58 | 16·4 |
| 34 | 17 | 3·0 | 7·1 | 116 | 16·4 |
| 15 | 11 | 4·25 | 14·2 | 232 | 16·4 |

area of application was important. We therefore examined the effect of changing the area to which the sensitizing dose was applied and studied the relationship between the concentration (dose per unit area) of the sensitizing dose and sensitization.

## METHODS

### *Subjects*

Healthy adult volunteers were recruited from the dermatology out-patient department. Individuals with a history of atopy, contact dermatitis or psoriasis were excluded. All subjects gave informed consent, and ethical committee approval was obtained for this study.

### *Sensitization*

Solutions containing 5, 2·5, 1·25 and 0·625 μg/ml of DNCB in acetone were prepared. A perspex template was made bearing holes to give the required areas (Table 1). The chosen circle was drawn on the volar aspect of the forearm with a fine ball-point pen. An accurately measured volume of the appropriate solution of DNCB was applied to the outlined area of skin with a micropipette. After the area had dried, it was occluded with polythene held in place for 48 h.

### *Experimental groups (Table 1)*

(i) To give the same concentration as 250 μg applied to the 3 cm diameter circle, 62·5 μg DNCB was applied to a circle of diameter 1·5 cm on seven subjects. This gave a dose per unit area of 35·4 μg $cm^2$.

(ii) In a previous study (Friedmann *et al.*, 1983) the dose which would sensitize 50% of subjects when applied on the 3 cm diameter circle was estimated as 116 μg, which gives a dose per unit area of 16·4 μg/$cm^2$. This was tested on 34 subjects. To investigate the effect of keeping the dose per unit area constant while varying the total dose, 15 subjects received double the total dose of DNCB on double the area and another 22 received half the total dose on half the area (Table 1).

### *Challenge*

The same technique for challenge was used for all groups. Challenge was performed 4 weeks after the application of the sensitizing dose. Each subject received challenge doses of 3·125, 6·25, 12·5 and 25 μg applied in 10 μl volumes of the appropriate solutions on standard 10 mm foil-backed patch test felts (Al test) taped onto the volar aspect of the right forearm for 24 h. Forty-eight hours after application, the reaction at each challenge site was measured as increase in skin fold thickness with Harpenden skin calipers from which one spring was removed.

### *Statistical analysis*

The responses to challenge in sensitized subjects are shown as plots of response (expressed as increase in skinfold thickness) against log challenge dose. The normal augmentation of responsiveness with increasing sensitizing stimulus is shown by plotting the responses to the 12·5 μg challenge dose against log sensitizing dose for the five groups who received sensitizing doses of 62·5 up to 1000 μg on the 3 cm diameter circle (normal subjects reported by Friedmann *et al.*, 1983). Log dose response curves were fitted by linear regression. Slopes were compared by analysis of variance. The proportions of subjects who became sensitized in each group were compared by a Chi-squared test.

## RESULTS

### *Effect of 62·5 μg applied to a 1·5 cm diameter circle*

The sensitizing dose of 62·5 μg applied on the 1·5 cm diameter circle sensitized six out of seven subjects (89%), compared with two out of 24 (8%) sensitized when applied to a 3 cm diameter circle ($P < 0·01$).

The sensitizing dose of 250 μg applied to a 3 cm diameter circle gives the same concentration (35·4 $\mu g/cm^2$) as 62·5 μg applied to an area with a 1·5 cm diameter. These two doses sensitized similar proportions of subjects, 89% and 81% respectively (Table 1), and furthermore, the

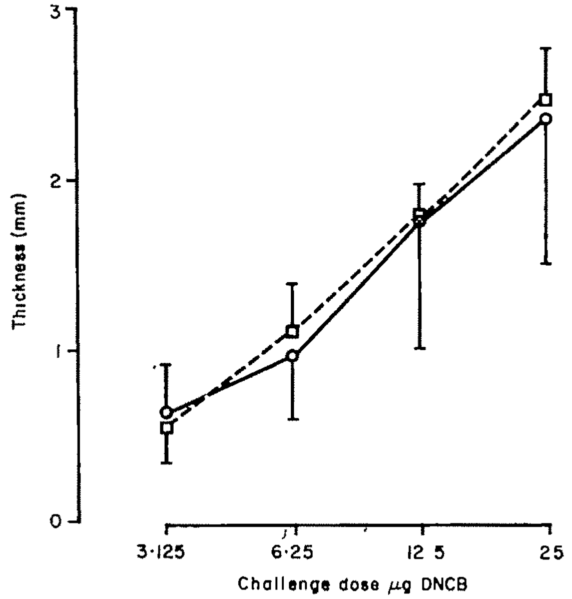

FIGURE 1. Responses in subjects all sensitized with the same dose per unit area, 35·4 $\mu g/cm^2$, of DNCB. Subjects succesfully sensitized with 250 μg on a 3 cm diameter circle: □– – –□, or with 62 5 μg on a 1 5 cm diameter circle: ○——○. Points are the means ± SEM.

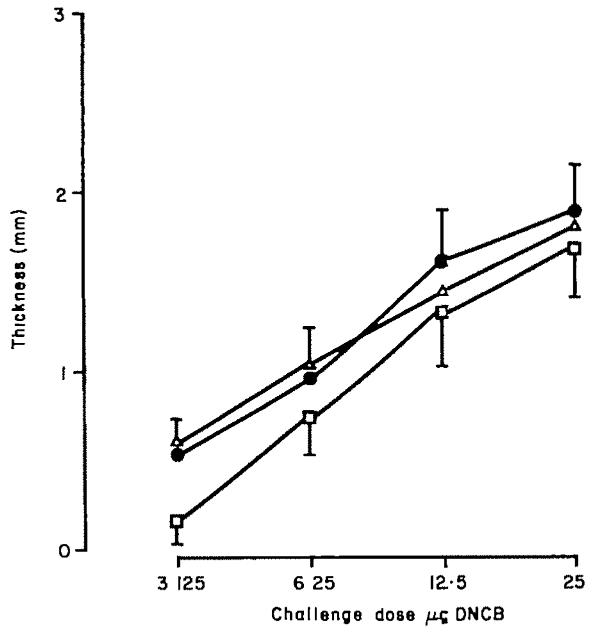

FIGURE 2. Responses in subjects all sensitized with the same dose per unit area, 16 4 µg/cm², of DNCB. Subjects succesfully sensitized on 14 0 cm². ●——●; 7·1 cm²: △——△; or 3 5 cm²: □——□. Points are means ± SEM.

responsiveness of sensitized subjects was virtually identical (Fig. 1). Thus, it seemed sensitizing concentration is a major determinant of the sensitizing potency of a given dose.

*Effect of changing the sensitizing dose whilst maintaining a constant concentration*

The dose of 116 µg applied to the 3 cm diameter circle was estimated as the 50% sensitizing dose and indeed, 17 of 34 (50%) subjects became sensitized. These subjects were compared with groups who received the same concentration (16·4 µg/cm²), but as double the dose (232 µg) on double the area, and half the dose (58 µg) on half the area (Table 1). The numbers of subjects who became sensitized were 10 out of 15 (66%) and 12 out of 22 (55%) respectively; the numbers are not significantly different ($P > 0{\cdot}5$).

The degrees of responsiveness of these three groups, as shown by the positions of the log challenge dose response curves, were not significantly different (Fig. 2), although they showed a tendency for response to increase with total dose. When the responses to a 12·5 µg challenge dose are plotted against total sensitizing dose (Fig. 3), the log sensitizing dose response curve for the three groups sensitized at a constant concentration has a slope of 0·21 (SE 0·35), which is not significantly different from 0 ($t_{36} = 0{\cdot}60$). On the other hand, when area is kept constant (so that doubling the total dose also doubles the dose/cm²), the log sensitizing-dose response curve has a slope of 0·42 (SE 0·18), which is significantly different from 0 ($t_{82} = 2{\cdot}33$, $P < 0{\cdot}05$). However, analysis of covariance shows that the two slopes are not significantly different from each other ($F_{1118} = 0{\cdot}29$).

## DISCUSSION

In this study, we have examined the effect of changing the dose and area of application of a sensitizer (DNCB) while maintaining a constant dose per unit area. A dose of only 62·5 μg applied to a small area actually had the same sensitizing capacity as four times that dose (250 μg) applied to four times the area. This suggests that the concentration of sensitizer per unit area is the major factor in determining sensitization.

In order to explore this further, we chose the 50% sensitizing dose and concentrations, which seemed likely to give the clearest answer since it was on the steep part of the dose-response curve. Indeed, for the three groups sensitized with the same concentration (16·4 μg/cm$^2$), the proportions of subjects sensitized did not differ significantly. Furthermore, the degree of reactivity of the sensitized subjects was not significantly different, although there was a trend of increased reactivity with increased total DNCB dose. To analyse this trend, the responses from each group at the 12·5 μg challenge dose were plotted against log sensitizing-dose (Fig. 3). The slope was not significantly different from zero and also appeared flatter than that from groups in which the sensitizing dose/cm$^2$ was doubled. However, the variability between patients was so great that the difference in slopes was not significant. It is now clear that a better choice of concentration would have been a higher one, nearer the middle of the log sensitizing dose response curve, e.g. 35·4 μg/cm$^2$, because a greater proportion of subjects would have been sensitized.

These findings can be explained only partly on the basis of percutaneous absorption. One of the main determinants of percutaneous absorption is the concentration of applied substance across the epidermis. At any concentration a given proportion of substance will penetrate. Therefore, doubling the amount and its area at that concentration should simply double the total

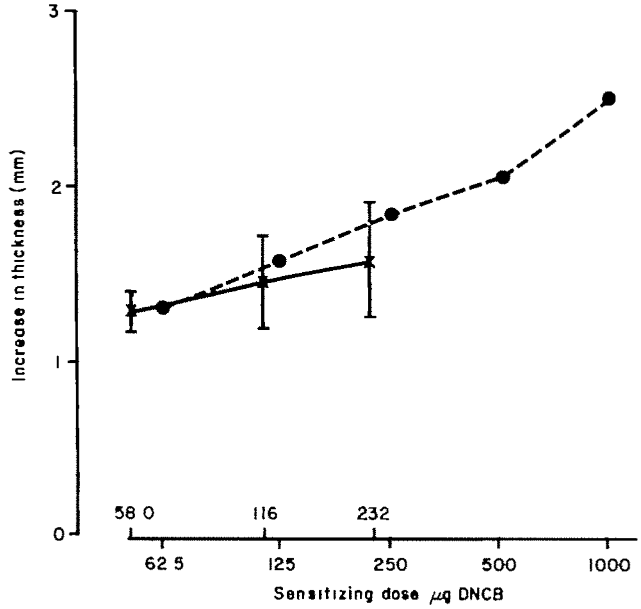

FIGURE 3. Dose-related augmentation of sensitivity. Response at 12 5 μg challenge dose plotted against log sensitizing dose. Subjects sensitized at a constant dose per unit area ×——×; or at a constant area ●---●. Points are means ± SEM.

amount that penetrates. On the other hand, doubling the concentration by doubling the total amount applied to a fixed area will result in a proportionally greater penetration of the substance so that more than double the amount will penetrate. The differences in the increments of sensitization induced by changing the concentration or by changing total dose at a constant concentration therefore must reflect the different increments of absorption of sensitizer.

The present findings indicate that the dose per unit area of a sensitizer is a major factor in the induction of contact sensitivity. The corollary is that it should be possible to reduce dose and area to an infinitesimal level and still obtain sensitization. The additional implication of this is that few Langerhans cells bearing many molecules of sensitizer are a more potent activating stimulus for the immune system than many Langerhans cells bearing few molecules of sensitizer. This possibility clearly requires further study.

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# Langerhans cells in human warts

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## SUMMARY

Seventy-six warts (15 plantar, 38 hand, 16 miscellaneous and seven anogenital lesions) taken from 55 patients, were studied by indirect immunofluorescence with monoclonal antibodies specific for T-cell subsets, Langerhans cells (LC) and HLA-DR antigen. The results were related to the presence of viral antigen. Approximately 80% of the lesions showed an infiltrate. Only 19 lesions contained helper/inducer or suppressor/cytotoxic T cells. The distribution of LC was abnormal in 65% of biopsies which contained LC in the dermis, and 29% were devoid of LC in the epidermis. Many lesions had reduced numbers of LC in the epidermis. The disappearance of LC from the epidermis was related to the presence of viral antigen, but not to the presence of particular T-cell subsets. Infiltrating cells were sometimes HLA-DR-positive, whereas basal cells did not express HLA-DR antigen, irrespective of the density of the infiltrate.

Both humoral and cellular immune mechanisms against human papillomavirus (HPV) particles and tumour cells have been documented in patients with warts. Studies have shown that mainly children and adolescents are infected by HPV, that many warts regress spontaneously after varying periods of time and that adults are less sensitive to re-infection (Cubie, 1972; Pfister & Zur Hausen, 1978; Briggaman & Wheeler, 1979).

Cell-mediated immunity plays an important role in wart regression (Thivolet, Viac & Staquet, 1982) and is initiated by a local cellular immune response. Although a mononuclear cell infiltrate is produced, as in other cutaneous tumours, little is known about the modulation of such reactions in papillomas of various clinical types (Chardonnet *et al.*, 1983; Löning *et al.*, 1983; Syrjanen *et al.*, 1984), except in plane regressing warts (Tagami, Oguchi & Ofuji, 1983).

In the present study, we investigated the presence of T-cell subsets and Langerhans cells (LC) in a series of human papillomas. We used specific monoclonal antibodies to determine the distribution of these cells in relation to HLA-DR and viral antigens in the lesions.

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## METHODS

### *Biopsies*

Seventy-six non-regressing papillomas were taken from 55 patients aged 11–70 years. From nine patients, two to six samples were taken from different locations. The papillomas were removed surgically, frozen in liquid nitrogen and stored at −20°C. They were usually excised 2–6 months after onset; a few had persisted for over 2 years. Various clinical types were studied: plantar ($n=15$), hand ($n=38$), genital area ($n=7$) and miscellaneous location ($n=16$) (arm, elbow, trunk, wrist and face).

### *Indirect immunofluorescence*

Frozen sections of 4 μm were prepared for indirect immunofluorescence as previously described (Chardonnet *et al.*, 1983).

### *Antibodies*

Monoclonal antibodies directed against specific antigens were used: OKT3, OKT4 and OKT8 from Ortho Pharmaceutical (Raritan, NJ, U.S.A.), specific for total circulating T lymphocytes, helper/inducer T lymphocytes and suppressor/cytotoxic T lymphocytes, respectively; OKT6 from Ortho Pharmaceutical, specific for LC; $BL_2$ from Dr J.Brochier, Lyon, France, specific for monomorphic HLA-DR antigen (Yonish-Rouach *et al.*, 1984). Viral antigen was detected with HPV antiserum raised in rabbits or in guinea-pigs against SDS-dissociated purified virus, dHPV (Jenson *et al.*, 1980).

### *Morphological studies*

Haematoxylin and eosin stained sections were examined.

### *Biochemical determination of the virus type in papillomas*

In all cases, total DNA was extracted . DNA was digested for 1 h at 37°C with a mixture of restriction enzymes (Hind II and Hind III; Boehringer, Mannheim, Germany) under conditions described by Orth *et al.* (1980). The DNA fragments were electrophoresed in 1% agarose gels. HPV types were determined after nitrocellulose transfer of fragments and Southern blot hybridization with $^{32}$P-labelled probes of HPV1 and HPV2 under non-stringent and stringent conditions.

## RESULTS

### *Local response of cutaneous and mucosal papillomas to HPV infection*

Seventy-six biopsies of non-regressing cutaneous and mucosal papillomas were examined for the presence of infiltrates, T-lymphocyte subsets, Langerhans cells, viral and HLA-DR antigens. The results are shown in Table 1.

Haematoxylin and eosin staining confirmed that all the biopsies studied had characteristic wart lesions. An inflammatory infiltrate was observed in the dermis of 61 of the 76 specimens (80%), irrespective of the location of the lesion. The phenotype of T3-positive cells was expressed only in 19 of 75 samples (25%). The numbers of lesions with T4- or T8-positive cells did not differ substantially; 13 of 75 (17%) and 17 of 75 (23%) respectively (Fig. 1a). The location of the lesion was not related to the distribution of the various cell types. When normal skin samples from similar locations were treated under identical conditions, no infiltration of T lymphocytes was detected.

TABLE 1. Local cellular immune response and presence of viral antigen in cutaneous and mucosal warts

| | Location and number of specimens | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hand | | | Plantar | | | Miscellaneous | | | Genital | | |
| | Total | Positive | (%) | Total | Positive | (%) | Total | Positive | (%) | Total | Positive | (%) |
| Presence of infiltrate | 38 | 30 | (79) | 15 | 12 | (80) | 16 | 12 | (75) | 7 | 7 | (100) |
| OKT3 positive cells | 38 | 7 | (18) | 15 | 2 | (13) | 15 | 6 | (40) | 7 | 4 | (57) |
| OKT4 positive cells | 38 | 6 | (16) | 15 | 2 | (13) | 15 | 3 | (20) | 7 | 2 | (29) |
| OKT8 positive cells | 38 | 7 | (18) | 15 | 2 | (13) | 15 | 5 | (33) | 7 | 3 | (43) |
| Presence of LC in dermis | 38 | 23 | (61) | 15 | 10 | (67) | 16 | 13 | (81) | 7 | 3 | (43) |
| Absence of LC in epidermis | 38 | 11 | (29) | 15 | 7 | (47) | 16 | 3 | (19) | 7 | 1 | (14) |
| Presence of viral antigen | 37 | 23 | (62) | 15 | 14 | (93) | 16 | 7 | (44) | 7 | 2 | (29) |

The morphology and the distribution of LC in papilloma lesions differed from those in normal skin. In some cases, LC had lost their dendritic shape and had become rounded. LC density was also altered. In 49 of 76 lesions (65%), LC were identified in the dermis (Fig. 1b); no LC were detected in normal dermis. The occurrence of LC in the dermis was not related to the location of the lesion. In 22 of 76 specimens (29%), no LC were detected in the epidermis. In most lesions, the number of LC was reduced (Fig. 1c), whereas normal epidermis contained large numbers of LC. The proportions of lesions with no LC in the epidermis varied according to location, the lowest being in genital lesions (1 of 7; 14%) and miscellaneous locations (3 of 16; 19%) and the highest in plantar warts (7 of 15; 47%).

Viral antigen (Fig. 1d) was present in 46 of 75 lesions (61%). The highest incidence was observed in plantar warts (14 of 15; 93%) and the lowest in genital lesions (2 of 7; 29%).

Viral DNA type 1 or type 2 was detected in most lesions. Only seven samples did not contain any viral DNA. Three others reacted under non-stringent conditions and their precise type was not determined. In some cases (six hand warts and five miscellaneous warts), viral DNA was detected in the absence of viral antigen.

Among seven hand warts without any detectable viral DNA, six did not contain any T-cell subsets and in all of them LC in the epidermis were absent or reduced in number.

Cells bearing HLA-DR antigen were identified in the dermis in 16 of 25 lesions (64%) (Fig. 1e) in contrast to normal dermis which was always negative. Although only 25 biopsies were studied, occurrence of HLA-DR- positive cells appeared to be related to the papilloma location; genital 3 of 3 (100%), miscellaneous locations 5 of 5 (100%), plantar 2 of 7 (29%), hand 6 of 10 (60%).

In 14 of the 25 lesions (58%) no HLA-DR-positive cells were detected in the epidermis. The proportions of negative lesions varied with the papilloma location from 0 of 3 in genital lesions to 7 of 7 in plantar warts. No HLA-DR antigen was identified in wart keratinocytes. Of these 25 lesions, 19 (76%) contained viral antigen, compared with 46 of 75 (61%) overall.

### *Analysis of papilloma lesions containing T-cell infiltrates*

Among 61 biopsies showing an infiltrate on haematoxylin and eosin staining, only 19 samples expressed T3, T4 and T8 phenotypes (Table 2). The infiltrate was either mild or moderate. Among these 19 lesions, a large proportion showed T4- or T8-positive cells, 12 of 19 (63%) and 17 of 19 (90%) respectively. Both T4- and T8- positive cells were found in 11 specimens (58%).

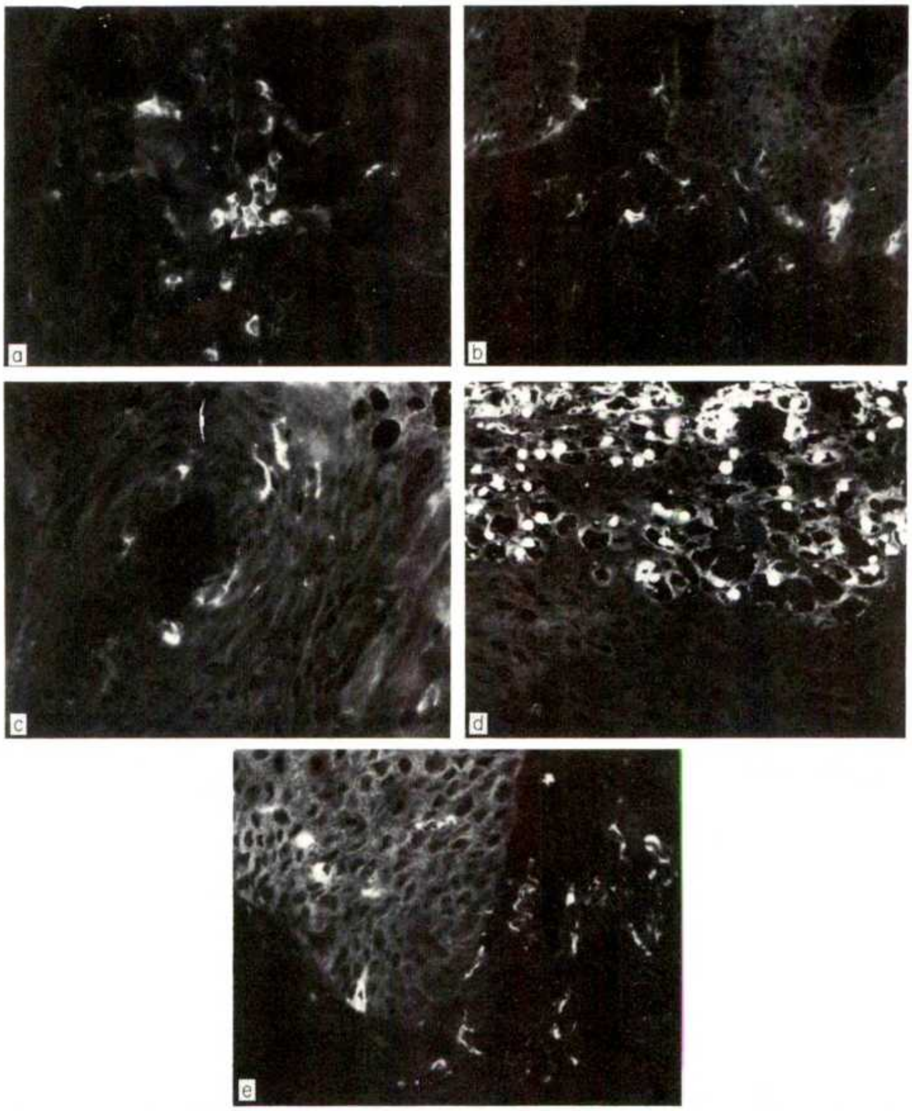

FIGURE 1. Immunofluorescence of frozen sections of human papillomas. (a) Dermal infiltrate with helper/inducer T cells in a hand wart; (b) abnormal presence of LC in the dermis of an elbow wart; (c) low density of LC in the epidermis of an elbow wart; (d) viral antigen in plantar wart detected with rabbit antiserum raised to SDS-dissociated purified human HPV1 virus; (e) HLA-DR antigen in dermis and epidermis of hand wart.

This percentage was higher than the percentages of lesions containing only T4 or T8-positive cells, 1 of 19 (5%) and 6 of 19 (32%) respectively. One specimen which reacted with OKT3 antibody failed to react either with OKT4 or OKT8 antibodies.

LC were present both in the dermis and the epidermis of 12 of 19 (63%) lesions; they predominated in biopsies with T8-positive cells (11 of 17; 65%). A large number of biopsies (14 of 19; 74%) contained LC in the dermis, whereas in 5 of 19 biopsies (26%) no LC were detected in the epidermis.

TABLE 2. Analysis of papilloma lesions with T-cell infiltrates

| | | Monoclonal antibodies* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | OKT6 | | BL2 | | Viral | | |
| Location | Patient no. | OKT3 | OKT4 | OKT8 | D | E | D | E | antigen detected | HPV type | Histology** |
| Hand | 1 | + | + | + | + + | + + | + + | + | (+) | 1 | + + + |
| | 2 | (+) | − | (+) | − | + | | | + | 2 | + |
| | 3 | + | (+) | + | − | − | | | (+) | 2 | + + |
| | 4 | (+) | (+) | (+) | + + + | + | + | (+) | + | 2 | (+) |
| | 5 | (+) | (+) | (+) | + + + | + | (+) | − | (+) | 1 | + |
| | 6 | (+) | + | + | + + + | + + | | | (+) | 1 | + + |
| | 7 | + | + | + | + + + | + + | − | + | (+) | 2 | + + |
| Plantar | 8 | (+) | (+) | (+) | + | − | | | + + | 1 | (+) |
| | 9 | + + | + + | + + | + + + | − | | | + + + | 1 | + + |
| Elbow | 10 | (+) | (+) | (+) | + + | + + | | | − | 2 | + + |
| | 11 | + | − | − | + | (+) | | | − | 2 | + |
| Face | 12 | + | − | + | + + | + + | | | + | 2 | + |
| Dorsum of foot | 13 | (+) | − | (+) | (+) | + | + | + | − | 2 | + + |
| Knee | 14 | (+) | − | (+) | + + | + + | + + + | + + | + | 2 | + + |
| Back | 15 | (+) | (+) | (+) | (+) | + + | | | (+) | 1 | + + |
| Genital | 16 | (+) | − | + | + + | + + | + | + | + | 2 | + + |
| | 17 | (+) | (+) | (+) | − | (+) | | | + | − | + |
| | 18 | (+) | − | + | − | − | | | (+) | 2 | + |
| | 19 | + | + | − | − | + + | + | + | − | 2 | + + |
| Normal skin | | − | − | − | | + + | | + + | − | | − |

* Immunofluorescence: (+), few cells weakly positive; +, few cells strongly positive, + +, many cells strongly positive; −, no staining. D = dermis, E = epidermis.

** Histology: (+), few mononuclear cells; +, slight infiltration; + +, moderate infiltration; + + +, intense inflammatory reaction.

The infiltrate did not stain consistently with HLA-DR antibody; some of the LC did not express this antigen. No basal epithelial cells were HLA-DR positive.

Viral antigen was largely associated either with the presence of T4- or T8-positive cells, 10 of 12 samples (83%) and 15 of 17 samples (88%) respectively, and was present mostly in lesions with LC in the dermis, 11 of 15 (73%).

HPV DNA type 1 was detected in six samples and type 2 in 12. One genital biopsy contained another type.

### *Correlation between the various parameters of the local immune response and the viral infection*

The presence of an infiltrate on haematoxylin and eosin staining was found in a large proportion of lesions which contained viral antigen (35 of 46; 76%) and of those which did not (25 of 29, 86%). Mononuclear cells were detected in a small number of biopsies. The T8 + cell subset was more frequent in lesions with viral antigen (15 of 46; 33%) than in those without (2 of 27; 8%). The absence or reduction of LC in the epidermis was not associated with the presence of an infiltrate but was more frequently observed in biopsies with viral antigen. The percentage of lesions without LC in the epidermis was higher when the viral antigen was present (18 of 22;

82%) than in negative biopsies (4 of 22; 18%). A similar relationship appeared between viral antigen and the presence of HLA-DR-positive cells in the epidermis.

## DISCUSSION

Our study of wart lesions has shown a mild or moderate local cellular immune response. Non-regressing cutaneous and mucosal papillomas were accompanied by an infiltrate, irrespective of their location. The lesions were characterized by the presence of T lymphocytes in the infiltrate, depletion of LC in the epidermis associated with the presence of viral antigen, presence of LC in the dermis and inconsistent expression of HLA-DR antigen in the infiltrate.

A similar inflammatory reaction has been described in regressing plane warts, in which high densities of mononuclear cells were found and T8+ lymphocytes outnumbered T4+ lymphocytes (Tagami *et al.*, 1983; Tagami, Oku & Iwatsuki, 1985). In two cases of cutaneous warts, an increase of T cells has been reported in which there was a predominance of helper/inducer T cells (Gatter *et al.*, 1984b). In our series, no T-lymphocyte subset predominated either in cutaneous warts or in mucosal warts infected with HPV type 1 or type 2.

The migration of LC from the epidermis to the dermis was characteristic of most of the lesions in the present study. A decrease of LC in the epidermis was also observed by Gatter *et al.* (1984b) in two cases of cutaneous warts. As LC are known to be effective antigen-presenting cells, they may have a role in activating T cells (Katz *et al.*, 1985). However, the mechanism by which LC present viral antigen to lymphocytes remains obscure. No viral particles have been found by electron microscopy (unpublished data). It is notable that the deletion of LC in the epidermis was frequently associated with the presence of viral antigen.

HLA-DR antigen is commonly observed in immunocompetent cells including B cells, activated T cells, macrophages and LC. Keratinocytes acquire this antigen in pathological states involving an intense inflammatory reaction (Gatter *et al.*, 1984a; Smolle, 1985). In papilloma specimens, HLA-DR antigen was expressed by only a few cells in the infiltrate. The epidermis was frequently HLA-DR negative, due either to LC depletion or to a loss of HLA-DR expression by LC. Keratinocytes failed to react with anti-HLA-DR antibody, probably because the inflammatory reaction was too weak, or because of the absence of a soluble factor such as $\gamma$-interferon, produced in the mononuclear cell infiltrate and responsible for HLA-DR expression (Volc-Platzer *et al.*, 1984). Hand warts have been found to be HLA-DR-negative in other studies (Gatter *et al.*, 1984a).

Taken together, our findings indicate that the inflammatory infiltrate in cutaneous and mucosal papillomas does not depend on the type of human papillomavirus or on the clinical type of papilloma. The infiltrate was mild or moderate irrespective of location. The amount of viral antigen produced by keratinocytes influenced the LC distribution in the tissue. This *in situ* immunological analysis is valuable for identification of cells which cannot be distinguished histologically.

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# The effect of thalidomide on arachidonic acid metabolism in human polymorphonuclear leukocytes and platelets

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## SUMMARY

The effect of thalidomide on the metabolism of arachidonic acid by purified washed human platelets and polymorphonuclear leukocytes was examined. The drug was found to be without effect under the conditions used. The action of thalidomide cannot therefore be attributed to a direct inhibition of prostaglandin or leukotriene biosynthesis.

Thalidomide is a potent anti-inflammatory agent which has been shown to be of value in severe orogenital ulceration (Jenkins *et al.*, 1984) and erythema nodosum leprosum (Sheskin, 1980), two conditions in which a neutrophil infiltrate is a prominent histological feature (Barnhill & McDougall, 1982; Graykowski *et al.*, 1966). Some of the products of arachidonic acid (AA) metabolism, such as leukotriene $B_4$ ($LTB_4$) from neutrophils and 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) from platelets, are known to have important inflammatory properties, especially neutrophil chemo-attraction (see reviews by Ford-Hutchinson & Chan, 1985; Dowd *et al.*, 1985). We therefore examined the effect of thalidomide on the metabolism of AA in human polymorphonuclear leukocytes (PMNL) and platelets, in an attempt to elucidate its mechanism of action.

## METHODS

Blood was taken from healthy donors who were not on any medication, and who had not taken any preparation containing aspirin during the preceding 10 days.

Suspensions of washed purified PMNL and platelets were prepared according to the standard methods used previously (Maurice, Bather & Allen, 1986; Maurice *et al.*, 1986). PMNL were

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resuspended finally in Dulbecco's buffer (phosphate-buffered saline containing 0·5 mM $MgCl_2$ and 0·9 mM $CaCl_2$, pH 7·3) to give $1{\cdot}5 \times 10^7$ cells/ml; purity was greater than 98%, and viability (assessed by trypan blue exclusion) was greater than 95%. Platelets were resuspended in 0·02 M Tris-HCl buffer, pH 7·6, containing 1 mM di-sodium EDTA, giving $5 \times 10^8$ cells/ml.

Thalidomide was purchased from Grunenthal, Stolberg, Germany. Because of its limited solubility in water, solutions of the drug were prepared in either water or water: dimethyl sulphoxide (50:50) to provide an adequate range of concentrations. The concentration of each solution was checked spectrophotometrically prior to use, according to the method of Green and Benson (1961). The final concentrations of thalidomide in the cell incubations were $3 \times 10^{-4}$ M, $5 \times 10^{-5}$ M and $3 \times 10^{-7}$ M. This concentration range spans the peak blood level of $6 \times 10^{-6}$ M measured after a single oral dose of 150 mg of thalidomide (Green & Benson, 1961). It was not possible to test concentrations of the drug greater than $3 \times 10^{-4}$ M because of its limited solubility.

Duplicate 0·5 ml volumes of cell suspensions were used in each experiment. The drug was added to one pair of tubes, and the solvent alone to the other, and the suspensions incubated in a water bath at 37°C with constant stirring. After 5 min, 0·1 μCi of $^{14}$C-AA, specific activity 58·4 mCi/mmol (Radiochemical Centre, Amersham, U.K.) was added, to give a final AA concentration of $3{\cdot}4 \times 10^{-6}$ M. In the case of the PMNL suspensions, calcium ionophore A23187 (Sigma Chemical Co., Poole, Dorset, U.K.) was added simultaneously (final concentration $3{\cdot}8 \times 10^{-6}$ M). Ionophore was not required for the platelet incubations. After a further 5 min, the reaction was terminated by acidifying the samples to pH 3·0 with 20 μl of 0·25 M HCl and then adding 0·75 ml of ice-cold water.

Samples were extracted twice with ethyl acetate (2 ml), dried under nitrogen, redissolved in a small volume of ethyl acetate, and each applied to one lane of a multi-lane thin layer chromatography (TLC) plate (Whatman LK6D). The plates were developed in toluene: dioxane: glacial acetic acid (65:34:1·5 by volume) at 4°C (Harvey & Osborne, 1983). In this system, a good separation of cylo-oxygenase and lipoxygenase products was achieved. The plates were then scanned using a Panax Thin Layer Scanner RTLS-1A, with argon quenching, to locate the peaks of radioactivity. The silica gel was scraped from these areas and counted in a liquid scintillation counter. The radioactivity (cpm) in each peak was expressed as a percentage of the total radioactivity recovered from each lane.

The major products were identified by comparing their $R_f$ values with those of authenticated standards applied to the same plate. The standards and their respective $R_f$ values in the solvent system used were prostaglandin $E_2$ ($PGE_2$, $R_f$ 0·25), thromboxane $B_2$ ($TXB_2$, $R_f$ 0·31) $LTB_4$ ($R_f$ 0·53), 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE, $R_f$ 0·65), 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT, $R_f$ 0·68) and 12- HETE ($R_f$ 0·72). The standards were a gift from Dr J.Walker, Lilly Research Centre, Windlesham, Surrey, U.K., except for the $PGE_2$ (Sigma Chemical Co., Poole, Dorset, U.K.) and $TXB_2$ (Radiochemical Centre, Amersham, U.K.).

## RESULTS

Incubation of purified PMNL suspensions with $^{14}$C-AA yielded radioactive products, identified on TLC as $PGE_2$, $LTB_4$ and its non-enzymic isomers, and 5-HETE. Platelet suspensions yielded $TXB_2$, HHT and 12-HETE. The percentage conversion of substrate into these products and the effect of the three different concentrations of thalidomide used is shown in Table I. The results for $5 \times 10^{-5}$ M and $3 \times 10^{-7}$ M thalidomide, and the corresponding control values, were compared using two-way analysis of variance, and the differences were not

TABLE 1. The effect of three different concentrations of thalidomide on the formation of AA metabolites by PMNL and platelet suspensions

| | PMNL | | | Platelets | | |
|---|---|---|---|---|---|---|
| Thalidomide | PGE$_2$ | LTB$_4$ | 5-HETE | TXB$_2$ | HHT | 12-HETE |
| Diluent alone ($n=5$) | 19·5 ± 1·8 | 16·3 ± 2·4 | 15·4 ± 2·0 | 32·1 ± 2·1 | 43·6 ± 4·0 | 12·2 ± 3·3 |
| $3 \times 10^{-7}$ M ($n=5$) | 19·6 ± 0·8 | 17·3 ± 4·5 | 14·5 ± 1·8 | 32·6 ± 2·3 | 43·3 ± 4·3 | 12·4 ± 4·5 |
| $5 \times 10^{-5}$ M ($n=5$) | 19·3 ± 0·7 | 15·8 ± 3·0 | 13·8 ± 1·3 | 31·7 ± 2·5 | 44·8 ± 3·8 | 12·9 ± 4·2 |
| Diluent alone ($n=3$) | 19·4 ± 0·2 | 15·5 ± 3·8 | 15·0 ± 0·1 | 20·9 ± 1·9 | 21·4 ± 0·6 | 37·0 ± 5·1 |
| $3 \times 10^{-4}$ M ($n=3$) | 17·1 ± 1·7 | 14·6 ± 1·2 | 13·2 ± 1·3 | 19·2 ± 2·0 | 20·6 ± 1·7 | 37·4 ± 4·1 |

Values represent percent conversion of substrate and are means ± SEM.
$n$ = number of experiments.

statistically significant. The results of a separate experiment using blood from different subjects and $3 \times 10^{-4}$ M thalidomide were compared with the corresponding control values using a paired *t*-test, and the differences were also not statistically significant.

## DISCUSSION

An early *in vitro* study on the mechanism of action of thalidomide demonstrated an inhibitory effect on the neutrophil chemotactic response to activated complement (Faure, Thivolet & Gaucherand, 1980). Miyachi *et al.* (1982) found that the drug inhibited generation of the oxygen intermediates $O_2^-$ and OH· by neutrophils without significantly affecting chemiluminescence. Subsequently, Barnhill *et al.* (1984) showed that thalidomide could depress phagocytosis of latex beads by neutrophils, but confirmed the lack of effect on chemiluminescence. Most recently, Powell *et al.* (1985) demonstrated that thalidomide had no significant effect in two *in vivo* models of leukocyte accumulation in rats, namely the carrageenan air pouch and pleurisy models. We have now shown that thalidomide, under the conditions used in the present study, has no effect on AA metabolism, in either PMNL or platelets. This is consistent with the findings of Hensby *et al.* (1984) who, by raising suction blisters on inflammatory skin lesions induced by tetrahydrofurfuryl nicotinate and analysing the contents, concluded that thalidomide has no direct action on the release or metabolism of AA in human skin.

The potent anti-inflammatory effects of thalidomide appear not to depend on a direct effect on AA metabolism, and further studies are required into its mode of action.

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# Human papillomavirus infections in a group of renal transplant recipients

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## SUMMARY

One hundred and twenty renal transplant recipients were investigated. Fifty-eight (48%) were found to have warts, 13 (11%) keratoses and six (5%) to have, or recently to have had cancers. The longer the time of immunosuppression, the greater the prevalence of warts; of those patients who had had their transplant for at least 5 years, 87% had warts. Those with a graft survival time of 10 years or more are at special risk of warts, keratoses and malignancy. Five (10%) of 50 women had genital warts, four of whom had internal lesions (vaginal, cervical or anal) and one developed a carcinoma of the vulva. These findings indicate the advisability of colposcopy for all female renal transplant recipients, a high risk group. Eighty-eight specimens from 42 patients were examined by DNA restriction enzyme analysis and cross hybridization for the presence and type of human papillomavirus (HPV). HPV DNA was detected in 66% of the warts examined, HPV2 and HPV4 occurring most often and HPV1 and HPV3 only infrequently. In sequential specimens from common hand warts of one individual, an HPV was found which could not be precisely identified but was related to HPV4. HPV16 was detected in a vaginal wart from one patient and an HPV6-related virus in a vulval wart of another. HPV DNA of an unknown type was demonstrated in one of 11 keratoses examined. With the probes used to examine the few samples of skin cancers available, HPV16 was found in a squamous cell carcinoma of the vulva, and faint bands from an unidentified type of HPV were detected in two squamous cell carcinomata from a patient's hand. One woman had plaque lesions morphologically and histologically resembling those found in epidermodysplasia verruciformis (EV). HPV5 was identified in these lesions. This is only the third reported case of HPV5, previously thought to be unique to EV, in a renal transplant recipient.

Renal allograft transplants have been performed since the mid-1960s. It is well recognized that the essential graft preserving immune suppression is associated with a number of complications, especially infections (Spencer & Andersen, 1979; L'Eplattenier *et al.*, 1980; Boyle *et al.*, 1984). Renal transplant recipients also suffer from an increased incidence of malignant tumours,

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including lymphomas and skin cancers (Penn, 1978; Kinlen *et al.*, 1979; Sheil *et al.*, 1979). Hardie *et al.* (1980) and Penn (1978) reported that the ratio of squamous cell carcinomas to basal cell carcinomas was reversed, although more recent work indicates that it is unchanged (Blohmé & Larkö, 1984; Boyle *et al.*, 1984).

This high incidence of malignancies has led to a number of theories. The finding that squamous cell carcinomas in patients with epidermodysplasia verruciformis (EV) harbour human papillomavirus (HPV) types 5, 8 and 14 (Orth *et al.*, 1980; Pfister *et al.*, 1981; Ostrow *et al.*, 1982), and the reported association of HPV16 and HPV18 with cervical neoplasia (zur Hausen, 1985), incriminate oncogenic viruses.

Lutzner *et al.* (1980) found two renal transplant recipients with an EV-like syndrome and demonstrated HPV5 DNA in their benign cutaneous lesions, and later in a skin cancer developing in one of them. Two questions therefore arise: whether infections with potentially oncogenic human papillomaviruses are a common feature in renal transplant patients, and if they are, whether this could link the high prevalence of warts and squamous cell carcinomas in this group.

Kremsdorf *et al.* (1984) noted that of the 24 HPV genomes characterized at that time, one half have been found only in EV patients and have not occurred in the general population. The reason is unclear, although Lutzner, Blanchet-Bardon and Orth (1984) suggest that it could be related to the immunodeficiency invariably present in EV patients.

The aim of the present study was to establish the types of HPV commonly found in the skin lesions of a group of renal transplant recipients in the south-east of Scotland, to search for an EV-like syndrome in these people and to check whether HPV genotypes so far associated with human malignancies could be detected in an area geographically distant from that where Lutzner *et al.* (1980) recorded their findings.

## METHODS

### *Patients*

One hundred and twenty renal allograft recipients were studied at the routine follow-up clinics at the Nuffield Unit, Western General Hospital, Edinburgh. A questionnaire was completed which included information regarding other medical conditions, drug regimens, wart history, hobbies, sun exposure at home and abroad and smoking habits. Patients were examined for the presence of warts, keratoses and skin cancers and any unusual skin lesion which might be attributable to HPV infection. Eighty-eight samples of lesional skin were taken from 42 patients by curettage, paring or biopsy, snap frozen in liquid nitrogen and stored at −70°C. Where the diagnosis was in doubt, part of the specimen was submitted for histological examination.

### *Virology*

*Source of human HPV DNA cloned in pBR 322.* HPV1, 2, 4 and 10 were kindly supplied by Dr T.S.Burnett, Department of Cancer Studies, University of Birmingham, U.K.; HPV3 and 5 by Dr R.Ostrow (Ostrow *et al.*, 1982, 1983); HPV8 by Dr H.Pfister (Pfister *et al.*, 1981); HPV6 by Dr M.Moar and HPV11 and 16 by Professor H.zur Hausen (Gissman *et al.*, 1982; Dürst *et al.*, 1983).

*Preparation of $^{32}$P-labelled HPV probes.* The host cells (*E. coli* HB101) were transfected, the cloned HPV DNA amplified, the host cells lysed by alkali and the resultant plasmid DNA purified on a caesium chloride density gradient (Maniatis, Fritsch & Sambrook, 1982). The HPV DNA was then nick translated and labelled with cytidine 5′-[α-$^{32}$P]triphosphate($^{32}$P dCTP) (Amersham International), according to the manufacturer's instructions.

*DNA extraction from parings, curetted and biopsied lesions.* Specimens were cut into small pieces when necessary, suspended in 1–2 ml 10 mM Tris, 10 mM EDTA, 0·5% sodium dodecylsulphate, pH 8, homogenized, and treated with proteinase K (Sigma), added to a final concentration of approximately 100 μg/ml. After overnight incubation at 37°C, large chromosomal DNA was precipitated at 4°C by the addition of 5 M NaCl to a final concentration of 1 M. For keratoses and cancers, this step was omitted. Samples were then phenol-extracted, and DNA precipitated with ethanol; dried and resuspended in 10 mM Tris 1 mM EDTA pH 8.

*Restriction analysis of the wart and tumour DNA.* 1–10 μg wart or 10 μg of tumour DNA was digested with restriction endonucleases following the addition of 0·2 μg RNAase I. The endonucleases used were a mixture of *Hin*d II and *Hin*d III (Boehringer Mannheim) for cutaneous warts; *Pst* I (Boehringer Mannheim) for genital material and B*am* HI (Northumberland Biolabs Ltd) for the EV plaque-like lesions. The reaction conditions were those recommended by the manufacturers. The fragments were separated by electrophoresis on a horizontal 1% agarose gel, run overnight at 30 mA, in Tris acetate buffer (pH 7·9). After staining with ethidium bromide, the bands were visualized on a UV transilluminator. The molecular weights of the fragments were estimated by reference to standard molecular weight markers.

*Southern blot hybridization.* Gels were depurinated and denatured (Maniatis *et al.*, 1982) and the DNA transferred to Genescreen membranes (New England Nuclear). The hybridization was carried out according to the manufacturer's instructions at either low (20% formamide), medium (33% formamide), or high (50% formamide) stringency, at 45°C. The filters were subsequently washed at low or high stringency before being exposed to X-omatic film in an X-omatic casette with an intensifying screen (Kodak). For the wart tissue, the activity of the probe was $10^4$–$10^6$ cpm/ml and for tumour tissue $10^6$ cpm/ml. DNA from cutaneous warts was hybridized sequentially with HPV1, 2, 3, 4 and 5 without stripping off the probes in between. Genital specimens were similarly hybridized with HPV6 and 16.

## RESULTS

### *Clinical findings*

One hundred and twenty renal transplant recipients were studied, 70 male and 50 female, aged from 11 to 72 years (mean 38·5 years). The graft survival time varied from 1 to 216 months with a mean of 45 months. The immunosuppressive regimen was prednisolone 5–20 mg daily, and azathioprine 50–200 mg daily in all but two patients who were receiving cyclosporin A instead of azathioprine. The prevalence of warts was 48% (58 of 120); there was no substantial difference between the sexes in the incidence of warts (males 50%, females 46%). In the group with a graft duration of 12 months or less, only 15% (5 of 34) had warts; of these, three (60%) had had their warts at the time of transplant. In the group whose graft survival was 5 years or more, the prevalence of warts was 87% (26 of 30). Only two (6%) of the 26 with warts had had them at the time of transplant.

There was no significant difference in the current dose of azathioprine between those with and those without warts (Chi square test). However, those with warts were currently receiving less prednisolone, 10 ± 2·8 mg daily, compared with the 13 ± 4 mg daily taken by those without warts.

The clinical types of warts found in the 58 affected patients are shown in Table 1. One type only was found in 47 (81%), of which 36 (75%) had common warts, nine (19%) plane warts and two (4%) genital warts. A mixture of types was found in 11 (18%), nine having two types and two having three types. Common warts occurred in all but one of these combinations, plane warts in four and genital and plantar warts each in three. Common warts, therefore, occurred in 46 (96%) of all cases, and plane warts in only 12 (25%). Genital warts occurred in six (10%). Plantar warts were the least common, present in only three (5%) of those with warts.

Genital warts occurred in five (10%) of the 50 women studied. One woman's warts were confined to the vulva (no colposcopy was performed). One woman had extensive vaginal warts and a third fulminating anogenital, vaginal and cervical warts. In these patients, the warts were of the condylomatous type. Two patients had the flat type of wart affecting the vulva, vagina and cervix. One of these developed a squamous cell carcinoma of the vulva. One man with a history of extensive anal warts had recently undergone surgery for the removal of a squamous cell carcinoma of the anus.

Keratoses, other than seborrhoeic keratoses, were found in 11% of patients (13 of 120). They occurred on areas of light-exposed skin, and were usually of the actinic type. The age of those affected ranged from 30 to 65 years (mean 51 years). Graft survival time was 1–18 years (mean 10 years). Four had outdoor occupations and a further four had spent time in sunny countries. Skin cancers were present in four (30%) of these patients with keratoses.

The overall prevalence of cancer was 5% (6 of 120), comprising the four skin cancers and two anogenital cancers. Full details of these cases are shown in Table 2. One woman had a basal cell carcinoma on the brow. She has subsequently died of an unrelated internal malignancy. Three men had multiple squamous cell carcinomas, nine in all (two patients with two and one with five). All four had multiple warts and keratoses with some degree of actinic damage to the skin. Strangely, only one had a history of prolonged sun exposure. The two anogenital cancers were also squamous cell carcinomas, each occurring in a wart-bearing area. These patients also had warts elsewhere.

One woman, aged 39, who had received a transplant 6 years previously, was found to have three unusual pink scaly plaques about 1 cm in diameter on her right forearm. These lesions resembled, both clinically and histologically, those described in epidermodysplasia

TABLE 1. Clinical types of warts found in the 58 renal transplant recipients

| Numbers of patients with | | | | | |
|---|---|---|---|---|---|
| One type of wart | | Two types of warts | | Three types of warts | |
| Common warts | 36 | Common and plane | 4 | Common, plane, plantar | 1 |
| Plane warts | 9 | Common and plantar | 2 | Common, plane, genital | 1 |
| Genital warts | 2 | Common and genital | 1 | | |
| | | Common and anal | 1 | | |
| | | Plane and genital | 1 | | |
| Totals | 47 (81%) | | 9 (15%) | | 2 (3%) |

**TABLE 2.** Skin and genital lesions in six renal transplant recipients with malignancies and the type of HPV found in those lesions examined

| | | | | Lesions | | | |
|---|---|---|---|---|---|---|---|
| Patient | Sex | Age (years) | Years since transplant | Site | Type | HPV | Comments |
| 1 | M | 49 | 5 | Neck | SCC | NE | |
| | | | | Chest | SCC | NE | |
| | | | | Neck, arms | Keratoses | Not detected | |
| | | | | Neck | Common wart | Not detected | |
| | | | | Face | Common Wart | Not detected | |
| | | | | Hands | Common wart | HPV1 | Three samples |
| | | | | Soles | Plantar warts | HPV1 | Five samples |
| 2 | M | 46 | 10 | Scalp | SCC | Not detected | |
| | | | | Scalp | SCC | NE | |
| | | | | Scalp | Keratoses | NE | |
| | | | | Hands | Common warts | HPV4 | |
| 3 | F | 57 | 14 | Brow | BCC | Not detected | |
| | | | | Arms | Keratoses | Not detected | |
| | | | | Hands | Common warts | Not detected | |
| | | | | Buttock | Common warts | HPV2 | |
| 4 | M | 40 | 19 | Scalp | SCC | NE | |
| | | | | Hands | SCC | Equivocal | HPV5 and 8 hybridization at low stringency only |
| | | | | Hands | SCC | Equivocal | |
| | | | | Back | IEC | NE | |
| | | | | Hands | IEC | Not detected | |
| | | | | Hands | Common warts | HPV2 | Different restriction pattern |
| | | | | Hands | Common warts | HPV2, HPV4 | |
| | | | | Soles | Mosaic warts | HPV2 | Four samples |
| | | | | Scalp, neck, arms, hands | Keratoses | Not detected | One sample examined |
| 5 | M | 41 | 7 | Anal | SCC | NE | |
| | | | | Anal | Genital warts | NE | |
| | | | | Hands | Common warts | HPV2 | |
| 6 | F | 35 | 12 | Vulva | SCC | HPV16 | |
| | | | | Vulva | Genital warts | NE | Specimen unsuitable |
| | | | | Vagina | Gential warts | NE | |
| | | | | Cervix | Genital warts | NE | |
| | | | | Arms | Keratoses | Not detected | |

BCC, basal cell carcinoma; IEC, intra-epidermal carcinoma; SCC, squamous cell carcinoma; NE, not examined.

verruciformis. She had no typical warts, keratoses or skin cancers, but has recently developed further similar lesions on one leg.

### *Virological findings*

Tissue was not available from all lesions noted, but 88 specimens were obtained from 42 patients. HPV was not detected in all the specimens examined. While the majority of HPV DNA could be typed on the basis of the restriction enzyme fragments (Fig. 1), all the samples were also subjected to Southern blot hybridization to confirm the result. The specimens fell into six categories:

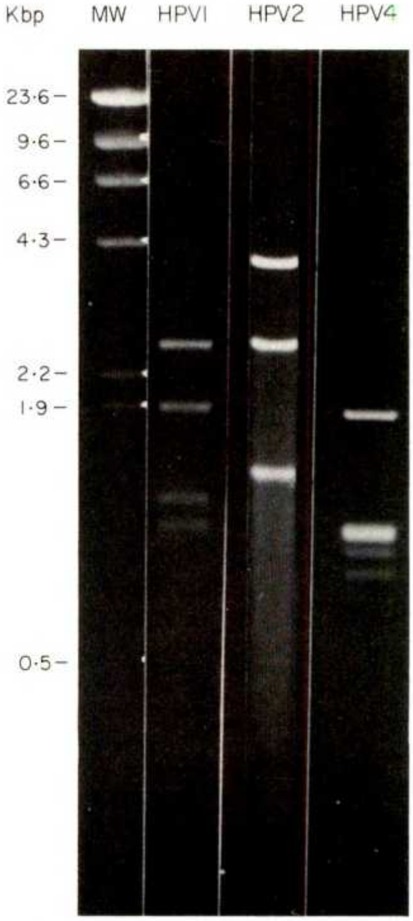

FIGURE 1. *Hind* II/*Hind* III restriction fragments of HPV1, HPV2 and HPV4 DNA.

(*i*) *Cutaneous warts from patients with no evidence of malignancies*. From the 28 patients in this category, 34 warts were examined. The types of HPV DNA identified following cleavage with *Hind* II and *Hind* III are shown in Table 3. Of the 24 common warts, eight yielded HPV2 and nine HPV4. In one instance where two specimens had been pooled, there was evidence of both HPV1 and HPV4. The DNA from the two plantar warts was identified as HPV2 in one instance and HPV4 in the other. Only two of the seven plane warts yielded identifiable HPV DNA, both being HPV3. Three individuals had two separate specimens each from a different site, in only one instance were both HPVs of the same type.

(*ii*) *Genital warts from patients with no evidence of malignancies*. Two specimens only fell into this category. One vaginal wart yielded a very small amount of DNA which was examined without endonuclease digestion. The three structural forms of the DNA were all shown to hybridize with HPV16 at high stringency, so this was most probably an HPV16 wart. The DNA extracted from a vulval wart in a second woman was digested with *Pst* I. The resultant fragments did not hybridize with HPV16 even at low stringency, but at medium stringency hybridized

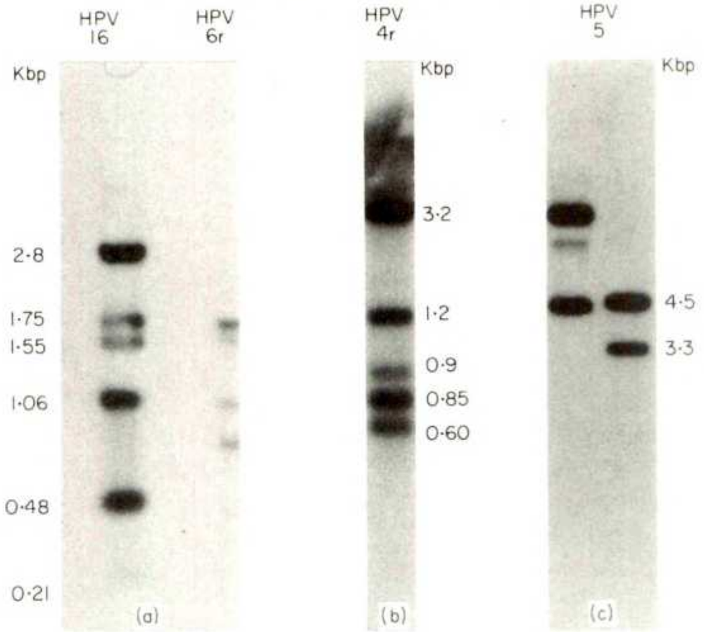

FIGURE 2. Autoradiographs of: (a) *Pst* I digests of DNA from an SCC of the vulva, hybridized with HPV16 at high stringency (left), and from a vulvar wart, hybridized with HPV6 at medium stringency (right) (HPV6r = HPV6 related). (b) *Hin*d II/*Hin*d III digest of DNA from a skin wart, hybridized with HPV4 at medium stringency (HPV4r = HPV4 related). (c) Undigested (left) and *Bam* HI digested (right) DNA from a plaque-like lesion, hybridized with HPV5 at high stringency.

with HPV6. However, the molecular weights of the fragments did not correspond with those of HPV6 (Fig. 2a). It was concluded, therefore, that this was an HPV6-related virus.

(*iii*) *Specimens from patients with malignancies.* The details of the types of HPV found in the lesions from the six patients with malignancies are shown in Table 2. Unfortunately, tissue was not available from all the lesions occurring in these patients. HPV1 only was found in the widespread warts of Patient 1. Tissue from his cancers was not available.

Patient 2, with a squamous cell carcinoma of the scalp in which no HPV DNA was detected, had HPV4 in his hand wart specimens. No HPV DNA was detected in the basal cell carcinoma of Patient 3 but HPV2 was found in her warts. The findings in Patient 4 were particularly interesting. HPV2 was found in the majority of his warts, but one gave DNA with a different restriction pattern and another yielded both HPV2 and HPV4. Both carcinomata from his hands yielded DNA, which gave faint bands on the autoradiograph following hybridization with HPV5 and HPV8 at low stringency. These bands did not coincide with any *Hin*d II/III fragments of standard HPV5 or HPV8, and no hybridization was detected at high stringency. No specimens from the anal warts and carcinomata of Patient 5 were obtainable as these were removed prior to the study, but HPV2 was isolated from his hand wart specimens.

The vulval carcinoma of Patient 6 contained HPV16 confirmed by Southern blot hybridization following *Pst* I digestion. Her keratosis did not yield HPV DNA. Unfortunately, the vulval wart specimen proved unsuitable for analysis.

TABLE 3. Types of warts found in renal transplant recipients showing no evidence of malignancy and type of HPV identified in each

| Number of patients | Number of samples | Type of wart | Number of samples in which HPV identified | | | | |
|---|---|---|---|---|---|---|---|
| | | | HPV2 | HPV3 | HPV4 | HPV1 and HPV4 | HPV not detected |
| 18 | 21 | Common | 6 | ... | 8 | 1* | 6 |
| 7 | 7 | Plane | ... | 1 | ... | ... | 6 |
| 1 | 2 | Common and common | 1 | ... | 1 | ... | ... |
| 1 | 2 | Common and plantar | 2 | ... | ... | ... | ... |
| 1 | 2 | Plane and plantar | ... | 1 | 1 | ... | ... |
| Total 28 | 34 | | 9 | 2 | 10 | 1 | 12 |

* Pooled specimens.

(*iv*) *Specimens from patients with multiple warts but no evidence of malignancies.* Two patients fell into this category. In the first, who had had an allograft for 10 years, five warts from different sites were examined but in no case could HPV DNA be detected using the normal probes for cutaneous warts. In the second, with an allograft for 14 years, HPV DNA was not found in every wart sample but two warts on her hands did yield HPV DNA. The *Hin*d II/III fragments from both hybridized only with the HPV4 probe, at low and medium but not high stringency. From Figure 2b it can be seen that the molecular weights of the fragments do not correspond to those of HPV4 (Fig. 1), and it was therefore concluded that this was an HPV4-related virus.

(*v*) *Keratoses from all groups of patients.* Only one of the specimens from the 11 keratoses examined yielded any detectable HPV DNA. Bands were found following hybridization with a mixed probe containing HPV1, 2, 3 and 4. There was insufficient DNA to repeat the experiment with the probes singly.

(*vi*) *Specimens from EV plaque-like lesions in one patient.* The DNA from the plaques found in the patient was examined by the Southern blot technique using an HPV5 probe. Both undigested and B*am* HI digested samples gave bands with HPV5 at high stringency (Fig. 2c). The molecular weights of the B*am* HI fragments were in accordance with those previously published (Orth *et al.*, 1980).

## DISCUSSION

This study was primarily concerned with the typing of human papillomaviruses found in renal transplant recipients. Nevertheless, some interesting clinical information was obtained. The finding that 48% of the patients suffered from warts is similar to previous reports (Spencer & Andersen, 1979; Boyle *et al.*, 1984). The effect of graft survival time upon the onset of warts and skin lesions, already observed by Sheil (1984), was well illustrated in this study, in which the prevalence rose from 15% at the end of the first year to 87% after the fifth year. This difference is in fact even greater as the presence of warts at the time of transplant represents a larger proportion of the warts in the early group than in the late group; 5% of patients in the early group had new warts, and 80% in the late group. Furthermore, those with keratoses, and in particular those with malignancies, also had a long graft survival time; when this exceeds 10 years transplant patients would seem to be at special risk and regular dermatological screening is advisable.

The effect of the dose of immunosuppressive drugs on the prevalence of warts was investigated. The dose of azathioprine did not appear to have any influence. This is in line with the findings of Lennard *et al.* (1985) in their skin cancer study that neither the current nor the cumulative dose was important. The observation that those with warts were taking less prednisolone gives a false impression. The dose of prednisolone is highest soon after grafting and lowest in those with stable grafts. As a high proportion of those with warts fall into the latter group, the figures are biased. Estimation of the cumulative dose, which was not attempted, might have been a better parameter. It is likely that the duration rather than the level of immunosuppression is important.

Some authors suggest that plane warts predominate in renal transplant patients (Pfister, Gross & Hagedorn, 1979). In the present study, however, common warts predominated, being present in 96% of all cases, compared with plane warts in only 25%. The low prevalence of plantar warts was interesting and may reflect the life style of the patients. A number of mixed wart infections were found. This is a known feature of epidermodysplasia verruciformis; however, the prevalence of mixed wart infections in the general population is not known.

Only recently has it been suggested that immunosuppression is a high risk factor in the development of genital warts and tumours (Schneider, Kay & Lee, 1983). The lack of information regarding the prevalence of genital warts in renal transplant patients may be due to lack of recognition or concealment by the patient, unless directly questioned as in the present study. Although colposcopy was not carried out routinely, so that some patients with vaginal or cervical warts may have been missed, five (10%) of the 50 women were found to have genital warts, and 25% of women with warts of any kind had genital warts. Four of the women had vaginal and cervical warts, one being unaware of the fact until examined. It is not known whether the fifth woman also had internal warts. One woman developed a rapidly growing squamous cell carcinoma during the course of the study. These findings emphasize the importance of a full examination including colposcopy for all female renal transplant recipients. There is now a proven association between genital warts and cancer (McCance *et al.*, 1983) and these patients are a high risk group.

Considering the virological findings, our inability to detect HPV DNA in a number of specimens could have been due to inadequate or unsuitable sampling. A shave biopsy may prove more successful, especially in the case of plane warts. In view of the mixed infections found in some pooled parings taken from warts in close proximity, samples from individual warts should be stored and examined separately. On the other hand, the inability to detect HPV DNA, expecially in keratoses and cancers, could have been due to the use of insufficient probes, although hybridization at low stringency should reveal even distantly related HPV genomes.

There is some variation in the nucleotide sequence homology—up to 50% difference on DNA : DNA hybridization is allowable—within an individual HPV type (Coggin & zur Hausen, 1979). In our study, HPV4 DNAs proved to be a homogenous group but there was heterogeneity of HPV2 subtypes, a fact reported by Gross *et al.* (1983) and Favre *et al.* (1982). In three instances, we failed to identify the HPV genotype precisely. One was the sample from a keratosis, where a mixed probe was used and there was insufficient DNA to complete the study with individual probes. In the other two, hybridization with the relevant probes was detected at medium stringency, but the molecular weights of the fragments were not in agreement with those of the published HPV types so that they could only be classified as related to those types.

The clinical status of our renal transplant recipients with skin cancer closely resembles the EV syndrome as described by Lewandowsky and Lutz (1922), of immunodeficiency, widespread warts and actinically provoked skin cancers. The patients had all been immunosuppressed for many years, had widespread warts and their cancers were on light-exposed skin which showed actinic damage even without prolonged exposure. The man with the intra-epidermal carcinoma on his back admitted to frequent sunbathing in the past. The synergistic effect of sunlight in the carcinogenesis of skin cancer in renal transplant recipients has been demonstrated by Boyle *et al.* (1984). The recent finding by Lennard *et al.* (1985) that transplant patients with skin cancer have increased levels of the azathioprine metabolite, 6-thioguanine nucleotide in their red blood cells, introduces a further interesting dimension to the aetiology of cancers in these patients, but does not exclude a role for the carcinogenic types of HPV. Unlike the situation in EV in which there is a multiplicity of viral types, in particular those with malignant potential, HPV5, 8 and 14, the HPV types found in our group of patients were those found in the general population, HPV1, 2, 3 and 4.

HPV DNA was not positively detected in any of the non-genital tumour tissue examined, although in one patient there was thought to be weak hybridization with HPV5 and HPV8 probes under conditions of low but not high stringency. The experiment was repeated several times with the same result. The identification of HPV16 in tumour tissue from the patient who developed a vulval carcinoma is in line with the observation of McCance *et al.* (1983) and Gissman *et al.* (1984) that this type of HPV, togeter with HPV18, has a high oncogenic potential. This patient also suffered from prolonged and intractable pruritus vulvae, resulting in widespread excoriations. Chronic irritation is a well recognized synergistic factor in carcinogenesis (Underwood & Hester, 1971). The recent characterization in skin cancers of new types of HPV having a low degree of similarity with HPV16 by Scheurlen *et al.* (1985) and Ostrow *et al.* (1985) suggests that it would be worth including this and other probes in low stringency screening.

HPV DNA was only found in one of the keratoses studied and was of an unidentified genotype. In view of the discovery of virons resembling HPV in actinic keratoses by Spadbrow, Beardmore and Francis (1983), search for HPV DNA in this type of lesion should be continued using better sampling methods and by hybridization with additional probes.

The pink scaly plaques found in one patient conformed exactly with the clinical and histological appearance of such lesions occurring in 25% of the EV patients described by Jablonska (1984). This patient who received her transplant 6 years ago does not have typical warts or skin cancers at present but has recently developed further plaque lesions on her legs, and will be followed up carefully. The source of the HPV5 in this patient geographically isolated from any known EV patients, and in the two patients reported previously by Lutzner *et al.* (1980), remains a mystery. Further search for the virus in other patients in our group of renal transplant patients is important.

The present results suggest the need for further study of HPV in renal transplant recipients.

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***Case Reports***

# Erythroderma associated with mixed lymphocyte-endothelial cell interaction and *Staphylococcus aureus* infection

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## SUMMARY

We report 11 consecutive cases of erythroderma, a high percentage of which were associated with the growth of *Staphylococcus aureus* in cultures from blood, joint fluid or skin. In biopsies from all the patients we found close morphological associations between lymphocytes and endothelial cells, with some of the lymphocytes showing features of blastoid transformation to T helper lymphocytes. In extreme cases, sheets of T cells, including T helper lymphocytes, formed a syncytium with endothelial cells in the dermis. Marked capillary proliferation was noted both on light and electron miroscopy. We suggest that erythroderma is precipitated by antigens such as protein A, a potent T cell mitogen present on the cell surface of *Staphylococcus aureus*, or by drugs, such as phenytoin. These antigens induce antigen presentation by individual endothelial cells, leading to T helper transformation and lymphocyte proliferation. Endothelial proliferation resulting from lymphocyte-endothelial interaction results in the vascular proliferation associated with this syndrome.

Although many precipitating factors, ranging from psoriasis and contact eczema to drugs and pemphigus foliaceus (Rook & Wilkinson, 1979), have been noted to be associated with erythroderma or exfoliative dermatitis, the basic pathogenetic mechanisms underlying 'the red man syndrome' are currently unknown. The condition has been noted to regress in a few patients with the cessation of the causative drug or removal of the contact allergen. In the remaining cases, which form the majority, the erythroderma or exfoliative dermatitis becomes chronic, with a tendency to develop Pautrier's micro-abscesses and transform into mycosis fungoides many years later. We have had singular success in alleviation of both the pruritus and the erythema, which characterize the syndrome. We report here the 11 consecutive cases seen most recently and present light and electron microscopic findings.

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## CASE REPORTS

The patients were attending the out-patient dermatology clinics or were admitted to the medical wards of the Veterans Administration Medical Center, Sepulveda, California, U.S.A. Two patients were attending the dermatology clinics at Olive View County Hospital, Van Nuys, California, U.S.A.

### *Patient 1*

This patient, a 42-year-old Caucasian man, developed a dermatitis in the seborrhoeic areas (scalp, eyebrows, paranasal grooves, anterior chest and axilla). Over a 6-month period this had extended, despite topical steroid therapy, and, later, systemic therapy, to involve the whole body. He presented with the possible diagnosis of dermatomyositis, with marked proximal muscle weakness, but without Gottron's papules or the characteristic heliotrope erythema. Creatine phosphokinase was elevated at 620 mg/dl (normal < 180 mg/dl). Haematology was essentially normal apart from a leukocytosis of $15 \times 10^9$/l with a shift to the left. Muscle biopsies did not reveal inflammatory changes. He was mildly febrile, and blood and skin cultures grew *Staphylococcus aureus*, resistant to penicillin. He was treated with a 2-week course of intravenous oxacillin (methylphenyl-isoxazoyl penicillin, 1g every 6 h). He was also given prednisone, 60 mg daily orally, with no improvement in his muscle strength. However, when decreased muscle power resulted on increasing the prednisone dosage to 80 mg daily, the diagnosis of steroid myopathy was considered, and the steroids were gradually withdrawn. His muscle power rapidly improved over the ensuing weeks, and he was discharged with no therapy other than a petrolatum preparation for his mildly erythematous skin. Over the succeeding weeks, his erythema worsened, and he was readmitted when he developed a mild fever. Blood cultures again grew *S. aureus*. He was treated for 6 weeks with intravenous oxacillin (250 mg q.i.d.), and petrolatum for his skin, with resolution of his erythema after 5½ weeks. He had one further episode of mild erythema of his arms and neck a few weeks later. He was asked to stop using petrolatum on his skin and to moisturize it with mineral oil instead, and following a course of oral dicloxacillin for a further 6 weeks, his skin completely cleared, with no recurrence after a 3-year follow-up.

### *Patient 2*

This patient, a 56-year-old Caucasian man, had suffered from atopic dermatitis for many years, complicated by progressive erythema with severe itching for the past 3–4 years. He had been treated with topical steroids, both fluorinated and non-fluorinated, with much relief. He presented with a septic arthritis of the third metacarpophalangeal joint of his right hand. An aspirate of the joint fluid grew a penicillin-resistant *S. aureus*. His haematological investigations revealed a leukocytosis of $13{\cdot}8 \times 10^9$/l with a shift to the left (8% bands). He was admitted and treated with intravenous oxacillin (250 mg q.i.d.), with resolution of his erythroderma after 4 weeks of therapy. He was discharged on a further 4 weeks of oral dicloxacillin (250 mg (q.i.d.) and was able to be comfortable on bath oils and 1% hydrocortisone ointment. He still has occasional itching, but no recurrence of his erythrodermic episode.

### *Patient 3*

This patient, a 53-year-old Caucasian man, had chronic renal failure requiring haemodialysis three times a week. He developed an episode of acute erythema which had been present 3–4 weeks prior to his admission. One of his many blood cultures grew *S. aureus*. He received 6 weeks' treatment with intravenous oxacillin, with complete resolution of his erythroderma.

There has been no recurrence after a 2-year follow-up period. There was an initial leukocytosis ($1 \cdot 2 \times 10^9$/l).

*Patient 4*

This patient, a 68-year-old Caucasian man, had developed exfoliative dermatitis several months previously. He gave no past or family history of psoriasis or any other skin disease. Swabs from his skin produced a heavy growth of a penicillin-resistant *S. aureus*, for which he received vancomycin, with partial resolution of his erythroderma after 2 weeks. He was allergic to penicillin. After 4 weeks of therapy with vancomycin, he was changed to oral erythromycin (500 mg q.i.d.). He continued to improve, and after 8 weeks of antibiotic therapy, he was pronounced cured, and there has been no recurrence of his symptoms 2½ years later. Notable clinical features included severe itching prior to therapy, which improved after therapy, but only after several weeks, and a marked post-inflammatory hyperpigmentation after resolution of the erythroderma. Biopsies were taken from the skin of the trunk before and 2 weeks after vancomycin therapy. Skin swab cultures just prior to the cessation of antibiotic therapy had no growth.

*Patient 5*

This patient, a 42-year-old Caucasian woman, had had exfoliative dermatitis for the previous 18 months to 2 years. She had been biopsied on many occasions, and one of these biopsies had been reported to show the presence of Pautrier's micro-abscesses. A repeat biopsy by us, however, failed to confirm this finding. A heavy growth of a penicillin-resistant *S. aureus* was cultured from many areas of the erythrodermic skin from both trunk and limbs. She was treated with oral dicloxacillin (250 mg q.i.d.) with a partial resolution of her erythroderma, and a significant decrease in her pruritic symptoms after 4 weeks. When seen 4 weeks later, she was worse, with aggravation of her rash in areas where she had applied a lanolin containing bath oil. Mineral oil was substituted topically and oral dicloxacillin was continued with resolution of her erythroderma 4 weeks later. She was maintained on mineral oil alone with no recurrence of her erythroderma. Laboratory investigations revealed essentially normal haematology and biochemistry, apart from a mild leukocytosis of $12 \cdot 8 \times 10^9$/l, with a shift to the left (band neutrophils 7%). Skin cultures were negative for *S. aureus* at the end of therapy.

*Patient 6*

This patient, a 24-year-old female of Central American descent, had a past history of plaque psoriasis, which had resolved after application of a tar preparation and ultraviolet light exposure. She subsequently presented with erythroderma, accompanied by severe, generalized pruritus. Swabs from many areas of the erythrodermic skin produced a heavy growth of *S. aureus*. She was treated with oral dicloxacillin (250 mg q.i.d.) with partial resolution of the erythroderma after 4 weeks, and virtually complete resolution of the erythema after 10 weeks. The residual erythema appeared only on sun-exposed areas, and investigations revealed the presence of anti-double-stranded DNA antibody (titre 1:40) in her serum. Her residual symptoms improved with the use of sun-screens and beta carotene (150 mg daily), a free radical quencher. Marked post-inflammatory hyperpigmentation was noted.

*Patient 7*

This patient, a 35-year-old Caucasian man, had suffered from psoriasis for the past 20 years, and had been erythrodermic for 3–4 years. He had been found also to possess the MS phenotype for the alpha 1-antitrypsin deficiency gene (Heng, Moy & Lieberman, 1985). Swabs from various parts of his trunk and limbs gave heavy growths of a penicillin-resistant *S. aureus*. He improved with a 6-week course of oral dicloxacillin (250 mg q.i.d.), with virtual resolution of both pruritus and erythema at the end of this period. His only topical preparations had been petrolatum and bath oils. Haematological data before therapy revealed a mild leukocytosis of 12·1 × $10^9$/l, with a slight shift to the left (band neutropohils 6%). Negative skin cultures were obtained at the end of therapy.

*Patient 8*

This patient, a 23-year-old Black man, has had generalized atopic dermatitis for most of his life. Recently, he had been plagued by an intolerable generalized pruritus over his entire body. A moderate growth of *S. aureus* was obtained from swabs taken from various parts of his body. He was treated with oral dicloxacillin (250 mg q.i.d.) for 6 weeks with resolution of both the pruritus and the erythema. Skin cultures taken at the end of therapy were negative. He was able to maintain remission with bath oils and 1% hydrocortisone ointment.

*Patient 9*

This patient, a 63-year-old Caucasian man, had been treated with phenytoin sodium and phenobarbital for seizures for many years. Over the previous year, he had noted progressive pruritus, and the development of erythema over most of his trunk and limbs. Over the preceeding 2 months, he had noted increasing erythema and scaling, associated with a severe pruritus over the entire body. A diagnosis of erythroderma (exfoliative dermatitis) secondary to phenytoin was made, with complete resolution of the erythema and pruritus 3 months after the withdrawal of the drug.

He presented a year later with severe exfoliative dermatitis, associated with marked oedema of the entire body, including the face and eyelids. He had trouble opening his eyes. It was then discovered that he had been restarted on phenytoin sodium, and had been on this drug for at least 6 months. Swabs from the trunk and limbs produced heavy growths of *S. aureus*, and he improved dramatically, with virtual disappearance of his erythema after 2 weeks of oral dicloxacillin (250 mg q.i.d.) therapy. Phenobarbital was to have been substituted for phenytoin, but this was not done due to an oversight. However, he improved dramatically on dicloxacillin. Again, the notable clinical features included severe pruritus initially, and the development of marked post-inflammatory hyperpigmentation after resolution of the erythroderma. He was continued on oral dicloxacillin for a total of 6 weeks with complete resolution of his eruption, and with no evidence of recurrence when followed up 6 months later.

*Patient 10*

This patient, a 58-year-old Caucasian woman, had suffered from plaque psoriasis for several years. She presented recently with symptoms of severe itching and burning in one of the plaques on her leg, while another lesion on the elbow remained asymptomatic. A heavy growth of *S. aureus* was cultured from the skin, and she was started on oral dicloxacillin (250 mg q.i.d.). Her symptoms and marked erythema, which distinguished the lesion from the others that were asymptomatic, only resolved after 6 weeks of antibiotic therapy. After 8 weeks, dicloxacillin was discontinued as cultures from the skin were negative at that stage, and the severe itching and burning and intense erythema had largely subsided. Antipsoriatic therapy, which consisted of a tar preparation and ultraviolet light, was then reinstituted. Initial investigations revealed a mild leukocytosis of 12·2 × $10^9$/l, with a shift to the left (6% band neutrophils). Subsequent skin cultures revealed light growth of *S. aureus*, with the final culture, after resolution of the skin symptoms, being negative.

### *Patient 11*

This patient, a 45-year-old Caucasian man, recently developed generalized plaque psoriasis over his trunk and limbs, for which he received a topical tar preparation and UVB. On one of his out-patient visits, he was noted to be developing incipient erythroderma, with erythema involving both lower limbs, which was rapidly extending to involve his trunk. Severe itching characterized the erythrodermic areas, which, on culture, produced a heavy growth of a penicillin-resistant *S. aureus*. He was immediately started on oral dicloxacillin (250 mg q.i.d.), and, when seen 2 weeks later, the erythrodermic areas had virtually subsided, as had his symptoms of severe pruritus, leaving areas of post-inflammatory hyperpigmentation. The antibiotic was continued for a further 4 weeks with no further recurrence of the erythroderma. The patient was continued on his tar and UVB regimen, with subsequent clearance of his psoriasis.

## METHODS

### *Punch biopsies*

All 11 patients had at least one 4 mm punch biopsy of their erythrodermic areas prior to therapy. Patient 4 had a second biopsy performed after partial resolution of the erythroderma 2 weeks later.

### *Light and electron microscopy*

Each biopsy specimen was cut in half. One half was fixed in 10% neutral buffered 4% formaldehyde solution and processed for light microscopy. The other half was fixed in 2·5% glutaraldehyde buffered to pH 7·3 with 0·1% sodium phosphate, post-fixed in 1% osmium tetroxide, treated *en bloc* with tannic acid, dehydrated in alcohol and propylene oxide, and embedded in an epoxy resin mixture (Epon 812 and Araldite 502). Sections were cut on an ultramicrotome (Sorvall MT2B) with a diamond knife (Dupont), stained using a silver staining technique, and examined under an electron microscope (Philips EM 201).

## RESULTS

### *Light microscopy*

Skin sections stained with haematoxylin and eosin showed the presence of variable degrees of acanthosis, thinning or absence of the granular layer, and focal to widespread parakeratosis in all patients prior to therapy (Fig. 1). Mononuclear cells were frequently observed within the epidermis (Fig. 1), but there was no tendency to form Pautrier's micro-abscesses. Neutrophils were occasionally observed within the upper epidermis and stratum corneum, with Munro's micro-abscesses (Fig. 1) noted in five of 12 biopsies taken in the active stage of the disease. The dermal capillaries, particularly those within the superficial dermis, were increased in number. Many of these were lined by plump endothelium. The mixed cell infiltrate, which was mainly confined to the upper epidermis, appeared to be mainly, but not always, associated with superficial dermal blood vessels. However, in many areas, the infiltrate was more diffuse, with lichenoid features (Fig. 1), showing invasion into the epidermis in many areas (Fig. 1). Although skin swabs grew abundant *S. aureus* on culture, coccal forms were rarely observed in Gram-stained tissue. These were only observed within the layers of parakeratotic stratum corneum, and never within the living layers of the skin.

The biopsy taken from Patient 4 two weeks after treatment with vancomycin, revealed decreased density of the mixed cell infiltrate, as well as decreased acanthosis and parakeratosis.

TABLE 1. Clinical and pathological features in patients with erythroderma

| Patient No. | Age (years) | Sex | Underlying disease | Duration of erythroderma | Culture positive for *Staphylococcus aureus* | Leukocytosis cells/l | Munro's abscess | Endothelial cell lymphocyte interactions | Therapy |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 42 | M | Seborrhoeic dermatitis | 1 year | Blood | $15\cdot 0 \times 10^9$ | – | + | Oxacillin, dicloxacillin |
| 2 | 56 | M | Atopic dermatitis | 4 months | Joint fluid | $13\cdot 8 \times 10^9$ | + | + | Oxacillin, dicloxacillin |
| 3 | 53 | M | Chronic renal disease | 4 weeks | Blood | $12\cdot 2 \times 10^9$ | + | + | Oxacillin |
| 4 | 68 | M | None | 3 months | Skin | $10\cdot 2 \times 10^9$ | – | + | Vancomycin, erythromycin |
| 5 | 42 | F | None | 2 years | Skin | $12\cdot 8 \times 10^9$ | – | + | Dicloxacillin |
| 6 | 24 | F | Psoriasis (LE) | 4 weeks | Skin | $9\cdot 6 \times 10^9$ | + | + | Dicloxacillin |
| 7 | 35 | M | Psoriasis (generalized) | 3 years | Skin | $12\cdot 1 \times 10^9$ | – | + | Dicloxacillin |
| 8 | 23 | M | Atopic eczema | 3 months | Skin | $7\cdot 8 \times 10^9$ | – | + | Dicloxacillin |
| 9 | 63 | M | Seizure disorder | 2 months* | Not tested | Not measured | – | + | Stopped Dilantin |
|  |  |  |  | 3 weeks* | Skin | $13\cdot 2 \times 10^9$ | + | + | Dicloxacillin |
| 10 | 58 | F | Plaque psoriasis | 4 months | Skin | $12\cdot 2 \times 10^9$ | + | + | Dicloxacillin |
| 11 | 45 | M | Plaque psoriasis | 2 weeks | Skin | Not measured | – | + | Dicloxacillin |

* Two episodes; + = present; – = absent.

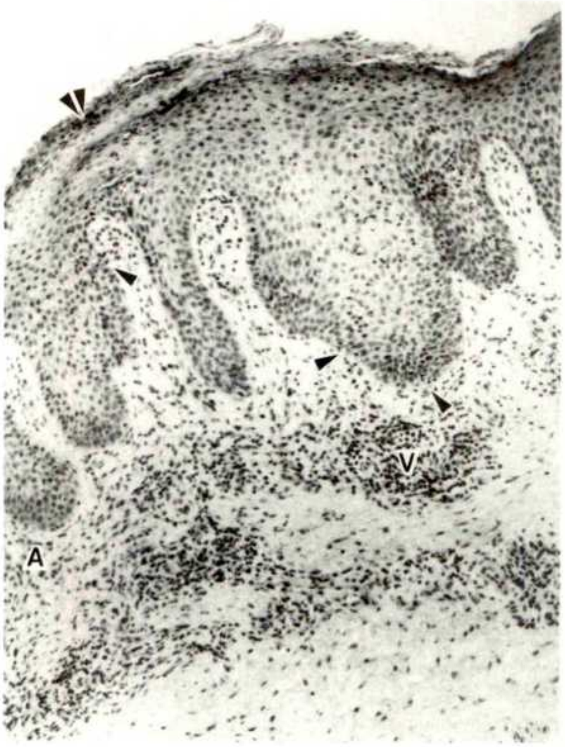

FIGURE 1. Photomicrograph of haematoxylin and eosin stained paraffin section from a patient with erythroderma (exfoliative dermatitis), showing moderate to marked acanthosis, loss of the granular layer, and widespread parakeratosis containing Munro's micro-abscesses (double arrow). The mixed cell dermal infiltrate is mainly perivascular (V), with lichenoid features (A). In many areas, the dermal infiltrate can be seen to invade the epidermis (single arrows). (Original × 120).

The capillary density appeared slightly decreased, although it is difficult to assess from a single biopsy.

### *Electron microscopy*

On electron microscopy, the most striking features included numerous associations of lymphocytes and endothelial cells, identified by the presence of large numbers of micro-vesicles (Fig. 2, inset) situated at the inner surface of the plasma membrane of the endothelial cell, and the presence of a large nucleus with few or no surface indentations and a thin rim of dense chromatin, interspersed by prominent nuclear pores. The nucleolus was usually prominent in proliferating endothelia. In addition, the endothelial cells had abundant cytoplasm, and possessed well developed rough endoplasmic reticulum and prominent mitochondria (Fig. 2). Those endothelial cells observed to be interacting with lymphocytes were not lined with basal lamina. The lymphocytes adjacent to these endothelial cells revealed convoluted nuclear membranes, and the characteristic chromatin pattern of lymphocytes, suggesting that these were probably helper T lymphocytes (Fig. 2). Other lymphocytes interacting with endothelial cells showed evidence of undergoing blastoid transformation, with large pale nuclei and

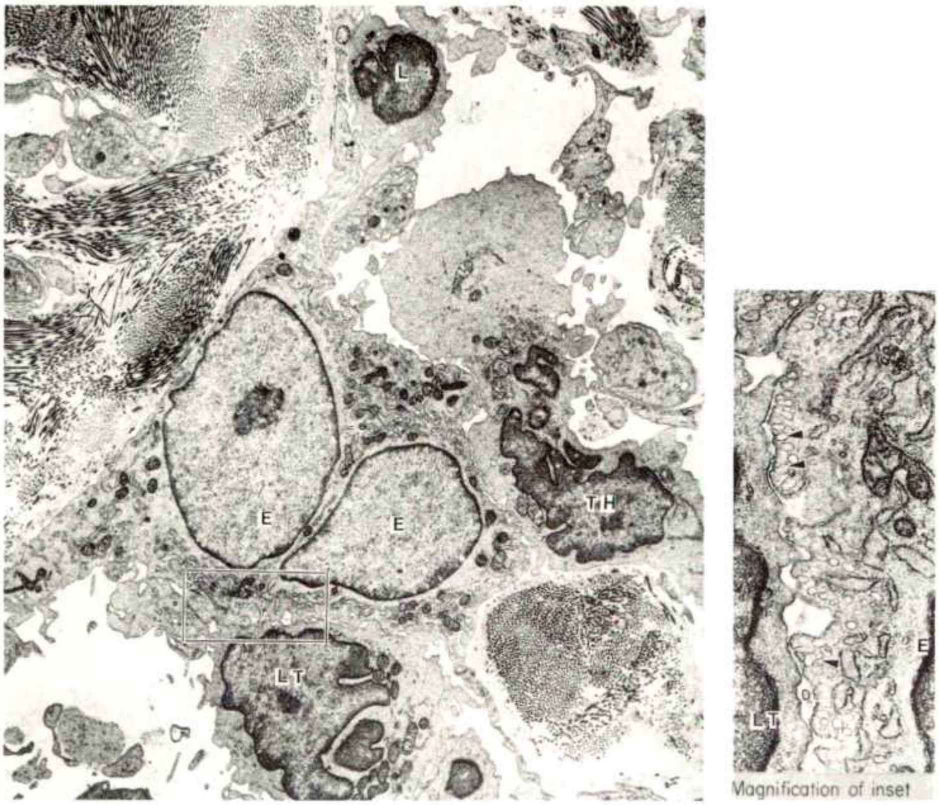

FIGURE 2. Electron micrograph of a biopsy from a patient with erythroderma showing the close association of an endothelial cell (E) with dermal lymphocytes. Note the marked invaginations of the nuclear membrane of the T helper lymphocyte (TH), the large, pale nucleus of the lymphocyte undergoing blastoid transformation (LT), compared with the untransformed lymphocyte (L). The endothelial cell (E) is identified by the presence of a large nucleus, with slight or no nuclear indentations, a thin rim of dense chromatin interspersed by prominent nuclear pores, a prominent nucleolus, and the presence of multiple microvesicular structures situated at the cell surface (inset, arrows). Note also the prominent rough endoplasmic reticulum and numerous mitochondria in the cytoplasm of the endothelial cell. (Original × 9000).

prominent nucleoli (Fig. 2). In some areas, the endothelial-lymphocyte interaction was associated with marked T lympohocyte proliferation (Fig. 3), consisting predominantly of T helper cells, with characteristically convoluted nuclei, and an occasional T lymphocyte showing features of undergoing blastoid transformation (Fig. 3). Interspersed among the T helper cells were endothelial cells, identified by the features described above (Fig. 3). In some patients, the lymphocyte proliferation was sufficiently dense to form a syncytium (Fig. 3).

Capillaries and post-capillary venules appeared to be markedly increased in number (Fig. 4), [4–12 per $10^4$ $\mu m^2$ grid space (200 mesh grids) in erythrodermic patients, and 0–2 in normal controls]. Many isolated endothelial cells, not lined by basal lamina, were observed in the

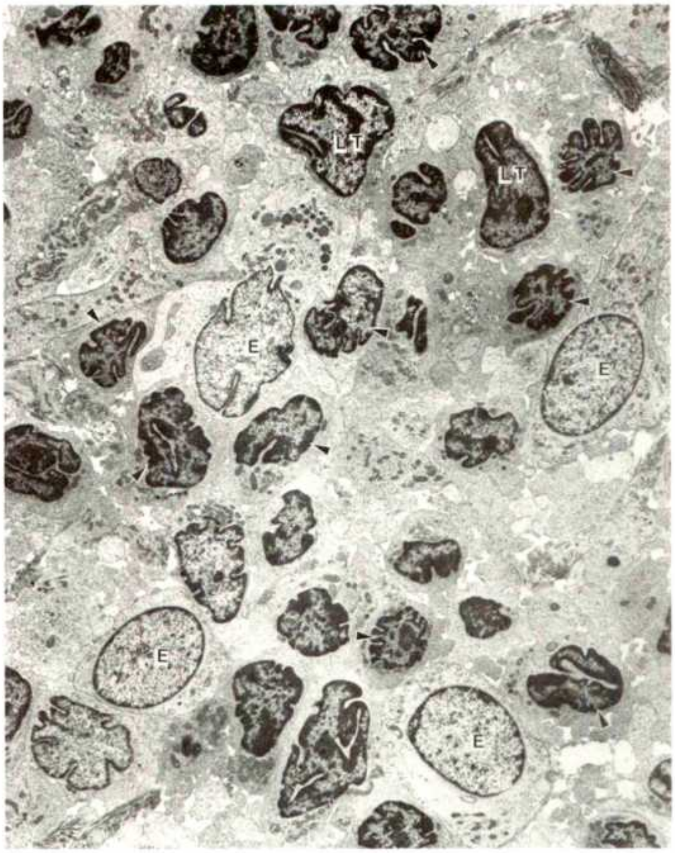

FIGURE 3. Electron micrograph of the dermal infiltrate, showing a syncytium of cells, made up predominantly of T helper lymphocytes, with characteristically convoluted nuclei (arrows), and endothelial cells (E). Note the presence of several T lymphocytes undergoing blastoid transformation (LT). (Original × 5000).

process of forming new capillaries (Fig. 4). Lymphocytes were frequently noted within the epidermis, as were neutrophils (Fig. 5).

The biopsy from Patient 4 two weeks after treatment with antibiotics revealed only an occasional presence of endothelial cell-lymphocyte interactions, and fewer lymphocytes. The syncytial appearance of the T lymphocytes and endothelial cells, as shown in Figure 3, was not observed, nor did the relatively sparse lymphocytes show the features of helper T lymphocytes. The capillaries did not appear to be markedly reduced in number, but actively proliferating endothelial cells forming new blood vessels were not observed.

## DISCUSSION

Our observations suggest that the erythema in exfoliative dermatitis is due both to vasodila-

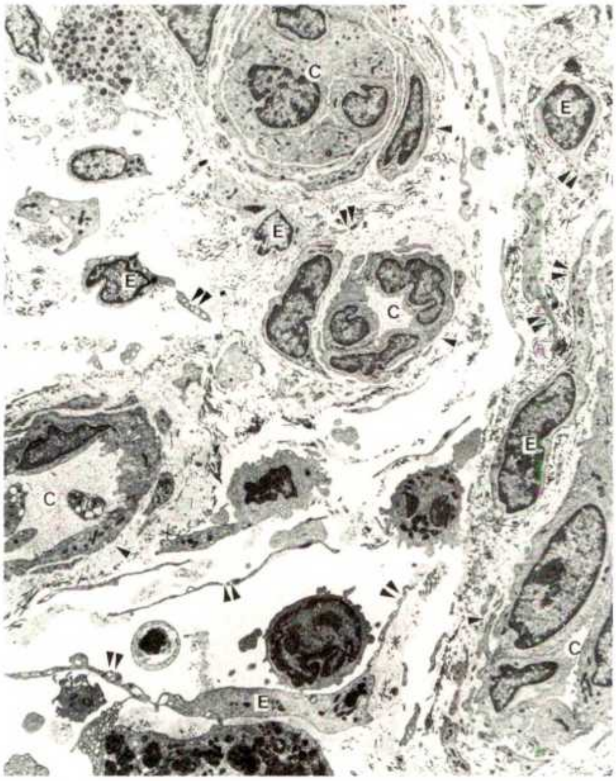

FIGURE 4. Electron micrograph showing the increased density of dermal capillaries (C). Note the migrating endothelial cells (E) with cytoplasmic processes (double arrows), unlined by basal lamina. Note also the attempt made by these migrating endothelial cells to form new capillaries. (Original × 3750).

tation and to vascular proliferation. Our findings that individual migrating endothelial cells do not possess a basement membrane (Fig. 4) supports previous observations that these actively migrating endothelial cells do not secrete a basement membrane until they become arranged in tubular capillary loops (Kalebic *et al.*, 1983). Thus, these actively migrating endothelial cells may be mistaken for macrophages, an observation previously noted in psoriasis induced by tape-stripping (Heng *et al.*, 1985).

The clinical features that characterize the erythrodermic syndrome associated with *S. aureus* include intense erythema, severe pruritus and a burning sensation, or both, and, occasionally, prominent post-inflammatory hyperpigmentation, especially in individuals who pigment easily. Symptoms may not subside for up to 6 weeks after therapy, and tend to recur with shorter courses of antibiotics. It is of interest that although the first episode of erythroderma in Patient 9 was thought to be due to phenytoin sodium, his second episode was probably due to *S. aureus* colonizing the skin, with a dramatic resolution of his severe erythroderma with marked exfoliation, after only 2 weeks of dicloxacillin.

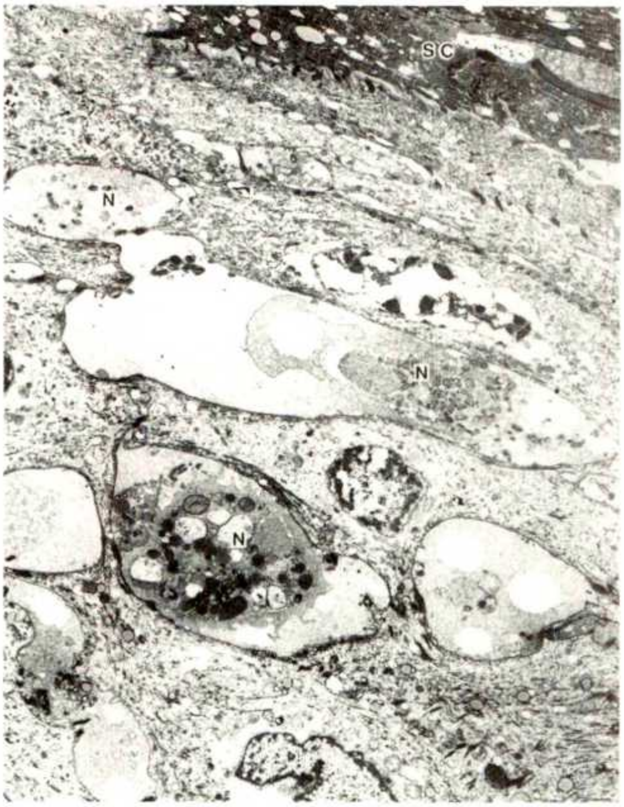

FIGURE 5. Electron micrograph of a biopsy from a patient with erythroderma, showing many neutrophils (N) within the upper epidermis. SC = stratum corneum. (Original × 7500).

None of the patients had visible pustules on their skin, nor were they febrile. Severe itch, rather than pain, was the most consistent symptom. Although some patients had a leukocytosis, with a slight shift to the left, this was mild, and may be related to the ability of protein A in *S. aureus* to initiate the generation of anaphylatoxins ($C3a$,$C4a$ and $C5a$), which induce neutrophil proliferation and chemotaxis. This may be a possible explanation for the high incidence of Munro's micro-abscesses found in five of our 11 patients. In contrast, other investigators have found a 25% incidence of Munro's micro-abscesses in their patients with plaque psoriasis (Krueger, 1982). In both light and electron microscopy, no staphylococci were found below the outer layers of the stratum corneum. We suggest, therefore, that the syndrome is caused, not by the infectivity of the organism, but by immunological reactions.

It has been observed that the ability of the *S. aureus* to activate complement depended upon a cell wall component, protein A (Langone *et al.*, 1984), and that complement activation did not depend upon the presence of living bacteria. It is of interest that protein A associated with bacteria, i.e. insoluble protein A, is an efficient mitogen for human B lymphocytes (Falkoff, Zhu & Fauci, 1982). On the other hand, soluble protein A is unable to induce proliferation of B cells, but is mitogenic for T lymphocytes (Romagnani *et al.*, 1978). It has been found that protein A can be eluted from the bacterial cell wall by the perfusion of plasma over the staphylococci, releasing soluble protein A (Gordon *et al.*, 1983), a discovery which has been utilized in the treatment of advanced malignancy (MacIntosh *et al.*, 1983). We suggest that the serum exuding onto the skin surface as a result of scratching could elute soluble protein A from staphylococci colonizing the stratum corneum, and that this could diffuse through the epidermis. Moreover, protein A has been found to impair opsonization by serum complement, thus delaying phagocytosis by neutrophils (Peterson *et al.*, 1977), further illustrating the complex interrelationships between host and parasite. Protein A and another antigen, enterotoxin A, are produced by *S. aureus* but not by *S. epidermidis*, which may account for our failure to associate *S. epidermidis* with erythroderma. Both protein A and enterotoxin A have been reported to be potent T cell mitogens (Jacob & Rose, 1983; Romagnani *et al.*, 1984).

The process whereby a T cell mitogen activates T lymphocytes is initiated by antigen presenting cells. These are members of the macrophage-monocyte series, which also include Langerhans cells (Rowden, Lewis & Sullivan, 1977; Klareskog *et al.*, 1977). It appears that the presence of the DR antigen on the surface of these antigen-presenting cells is essential to the performance of this function (Braathan & Thorsby, 1982). The T cell receptor consists of alpha and beta glycoprotein chains, the former recognizing antigen specificity and the latter, DR specificity. Recent work has shown that preparations of endothelial cells from unbilical veins were able to act as antigen presenting cells and support T cell proliferation in response to mitogens (Hirshberg, Bergh & Thorsby, 1980; Hirschberg, 1981; Burger *et al.*, 1981). Antigen presentation by endothelial cells has been shown to be HLA-DR restricted, as shown by inhibition by allosera reactive with HLA-DR (Hirschberg *et al.*, 1981). Our observations of the close association of lymphocytes and endothelial cells, as well as evidence of marked T cell proliferation in our patients with erythroderma, suggests the existence of cellular immunity in this syndrome. Our observation of an intimate interaction between these cell types has been noted *in vitro* by others (Hirschberg *et al.*, 1975). The presence of marked T cell proliferation with features of blastoid transformation and T helper cell differentiation (Fig. 5), would also support the suggestion of the involvement of a T cell mitogen in this process. We believe that in our patients, such a mitogen could either be an antigen, such as protein A from *S. aureus*, or a drug, such as phenytoin. Other less common antigens probably exist, but remain to be identified.

The culture of *S. aureus* from the skin in most of our cases (Table 1) suggests that this organism is the source of the T cell mitogen in many cases. We must then explain why eczema is not the predominant reaction rather than erythroderma, since Langerhans cells within the epidermis are the most logical cell to be involved as antigen-presenting cells. However, *S. aureus* has been found to have the capacity to inhibit manifestations of contact sensitivity by its ability to activate suppressor cells (Benedettini, DeLibero & Campa, 1984). We therefore believe that with the suppression of the normal contact mechanisms, the endothelial cell is stimulated to function as an antigen-presenting cell by the presence of soluble protein A, leading to both T cell and endothelial cell proliferation, with resultant pruritus and erythroderma. Similar findings in erythroderma associated with phenytoin supports the concept that immunological mechanisms, rather than infection, are involved in the pathogenesis of erythroderma.

We found that success in abolishing erythroderma depended upon the complete clearance of the erythroderma prior to the cessation of antibiotic therapy, an end point which also correlated with the absence of *S. aureus* in skin cultures. We observed that the abnormal (dermatitic) skin supported the growth of the organism, but once the skin returned to normal, it was no longer isolated. We therefore believe that the presence of the staphylococci on the skin in erythrodermic patients is not analogous to the carrier state in nasal carriers, but represents the source of the antigen responsible for the initiation of the immunological reactions.

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# Longitudinal melanonychia with spreading pigmentation in Laugier-Hunziker syndrome: a report of two cases

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## SUMMARY

We present two cases of Laugier-Hunziker syndrome with longitudinal melanonychia and Hutchinson's sign, associated with essential melanotic pigmentation of the mouth and lips.

Laugier and Hunziker (1970) reported five cases of essential melanotic pigmentation of the mouth and the lips. Two of these patients also displayed pigmentary changes in the nails. Sartoris *et al.* (1975) reported two cases, one of whom displayed a nail anomaly, and Laugier, Hunziker and Olmos (1977) described another patient with pigmented nails. We have encountered Laugier-Hunziker syndrome in 14 patients, eight of whom had nail involvement (Baran, 1979).

Recently, we observed two cases presenting with essential melanotic pigmentation of the inner cheeks associated with longitudinal melanonychia and periungual pigmentation involving all the fingers.

## CASE REPORTS

### *Case 1*

A 67-year-old female presented with multiple pigmented spots in the mouth and on the lips which had appeared 15 years previously. There were brownish, oval or round macules with a well-defined border affecting the palate, the cheeks and the mucous membrane of the lips. No perioral lesions were found. We noticed on the left thumb a 2 mm wide, longitudinal streak with pigmentation spreading from its proximal portion into the dorsal nail fold and its cuticle (Fig. 1). All the other fingers had dark round or oval spots anterior to the distal groove (Fig. 2). The patient was otherwise normal.

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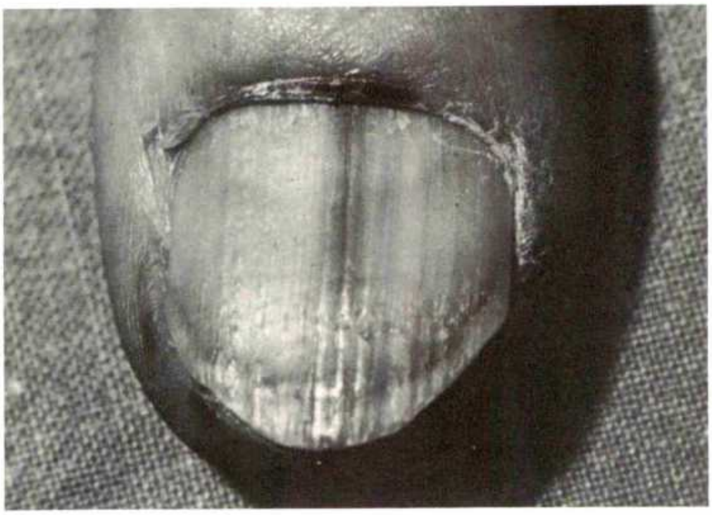

FIGURE 1. Case 1. Longitudinal melanonychia with pigment spreading into the proximal nail fold and its cuticle.

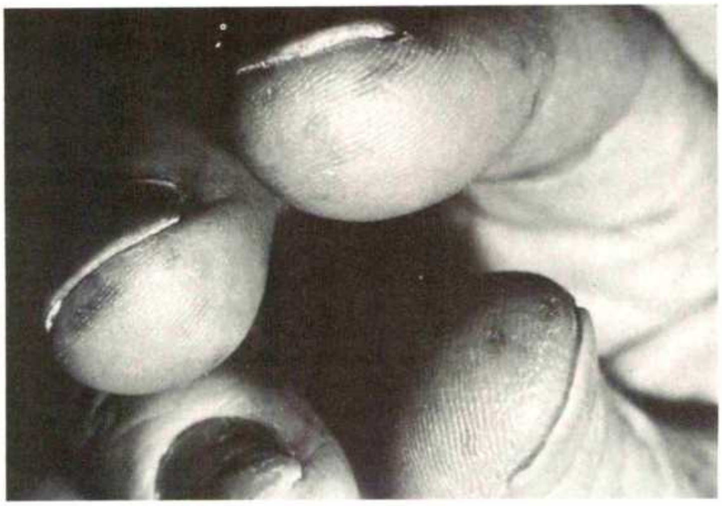

FIGURE 2. Case 1. Dark round and oval spots sited anterior to the distal groove.

In the biopsy taken from a dark spot on the pulp of the left index finger we noted, with Fontana staining, increased pigmentation of the basal cell layer of the epithelium spreading into the adjacent squamous cells as fine cytoplasmic granules (Fig. 3).

*Case 2*

This patient, a 57-year-old male, was seen in our clinic for hair loss. During his examination, we found that all his fingernails were affected by a longitudinal melanonychia accompanied by brownish spots in the periungual area also involving the proximal nail fold and the pulp (Fig. 4).

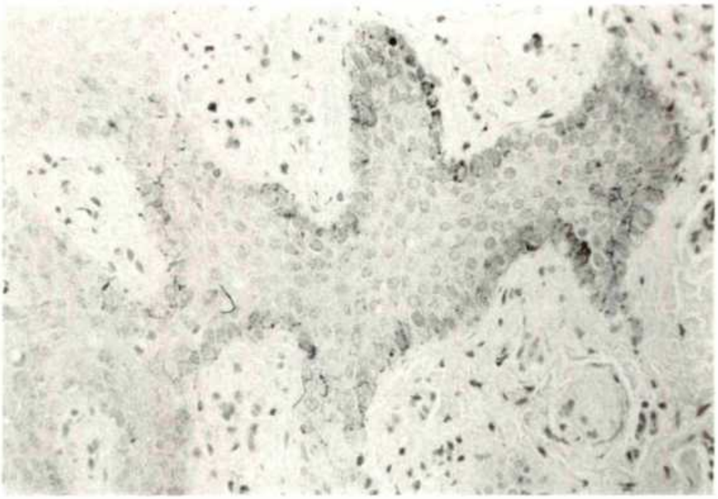

FIGURE 3. Case 1. Increased pigmentation of the basal cell layer of the epithelium spreading into the adjacent squamous cells as fine cytoplasmic granules.

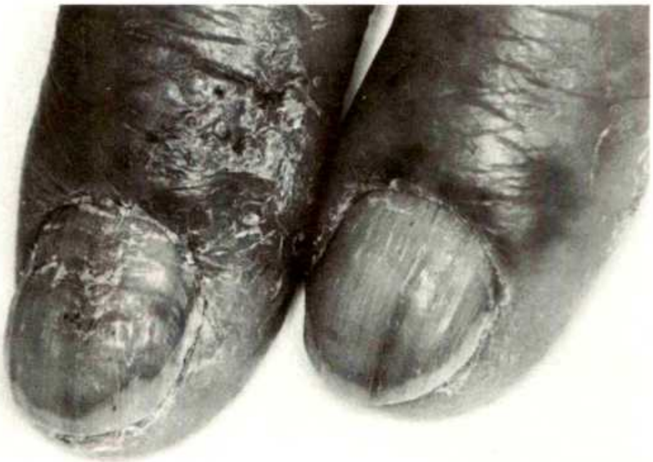

FIGURE 4. Case. 2. Longitudinal melanonychia involving all the patient's fingernails with brownish spots on the proximal nail fold.

On examining the mouth we found a lenticular slate macule on each cheek. The patient was not certain exactly when this pigmentary disorder first appeared, and was surprised by our interest in what he considered to be insignificant spots.

## COMMENT

Brown or black discoloration spreading from under the nail or proximal nail fold into the surrounding skin is termed Hutchinson's sign (Hutchinson, 1886). It has proved to be a valuable clue to the clinical diagnosis of malignancy (Kopf, 1981). Its presence in just one digit means that the entire nail apparatus must be removed (without prior incisional biopsy). This technique enables serial sections to be examined, which is particularly important in acral lentiginous melanoma in which histology may be difficult to interpret. Very rarely, periungual hyperpigmentation may be seen in melanocytic lesions other than malignant melanoma, such as in naevocytic naevus. Kopf and Waldo (1980) reported an interesting case with several recurrences of a longitudinal melanonychia showing some pigment in the proximal nail fold which lead to total excision of the nail apparatus and grafting. Histologically, most of the surgical specimens proved the lesion to be a recurring junctional naevus.

The appearance of Hutchinson's sign on a digit presenting with longitudinal melanonychia is distressing, except if more than one digit is involved, and it is accompanied by essential melanotic pigmentation of the mouth and the lips, characteristics of Laugier-Hunziker syndrome. In this syndrome, it should be stressed, there has been no evidence of local malignant changes at the sites of the spots on the mucous membranes or the longitudinal melanonychia in any case so far described.

Laugier-Hunziker syndrome should be added to the list of conditions in which periungual hyperpigmentation is seen in melanocytic lesions which are not malignant melanoma.

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# Congenital smooth muscle hamartoma: a case report

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## SUMMARY

We describe a case of congenital smooth muscle hamartoma with hypertrichosis and pseudo-Darier's sign, and suggest that it is a clinicohistopathological entity distinct from Becker's naevus.

Controversy exists as to whether the congenital smooth muscle hamartoma is a distinct clinicohistopathological entity rather than a form of congenital Becker's naevus. Becker's naevi usually occur during adolescence, and are characterized by hyperpigmentation and hypertrichosis, which increase with time. A proliferation of dermal smooth muscle bundles has been reported in some lesions (Haneke, 1979; Urbanek & Johnson, 1978). On the other hand, congenital smooth muscle hamartomas appear at birth and have hyperplastic dermal smooth muscle bundles with hypertrichosis. Hyperpigmentation may or may not be present.

We present here a case of congenital smooth muscle hamartoma to emphasize the clinical and histological features which differentiate it from a Becker's naevus and justify the acceptance of this disorder as a distinct entity.

## CASE REPORT

A healthy 2-year-old, Caucasian female was referred for evaluation of an enlarging asymptomatic congenital lesion on her left flank. Over the past year, her mother had noted the development of blonde hair within the lesion, in addition to redness and firmness after rubbing (pseudo-Darier's sign).

On examination, a 5 × 6 cm firm, slightly palpable plaque composed of small, discrete, flesh-coloured papules was present in the left suprailiac region. Hypertrichosis was noted throughout the plaque. Piloerection and erythema were produced within the lesion by minimal trauma.

Histopathological examination of a biopsy specimen from the superior border of the lesion showed a normal epidermis and many well defined bundles of smooth muscle fibres distributed

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randomly throughout the dermis. Some smooth muscle bundles were connected to the hair follicles (Fig. 1). Examination of a Masson trichrome stain confirmed the presence of smooth muscle bundles in the dermis.

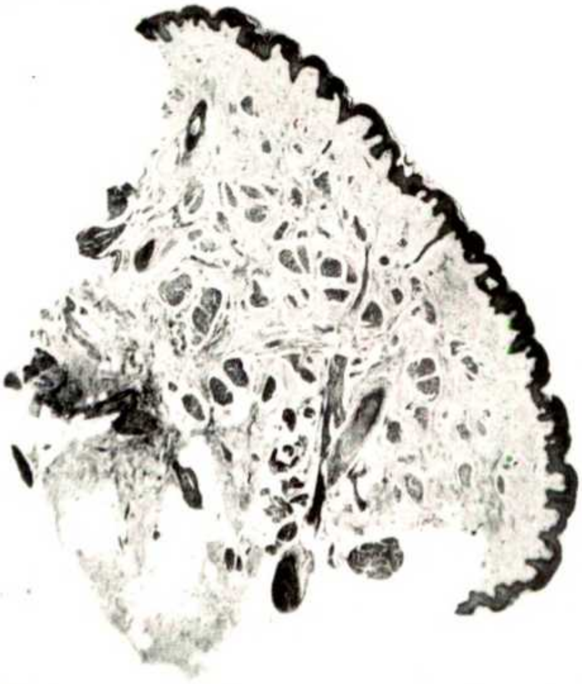

FIGURE 1. Biopsy from lesion on left flank. The epidermis is normal. There are well defined bundles of smooth muscle fibres scattered throughout the dermis. The hair follicles appear normal. (H & E; original × 10).

One year later, our patient had not developed hyperpigmentation within the lesion, and the hypertrichosis remained unchanged. Interestingly, the transient cutaneous elevation produced after mechanical stimulation (pseudo-Darier's sign) had diminished in intensity.

## DISCUSSION

Congenital smooth muscle hamartomas occurring independently of the classic Becker's naevus have been reported by others (Sourreil, Beylot & Delfour, 1969; Bonafe, Ghrenassia-Canal & Vancina, 1980; Karo & Gange, 1981; Chapel *et al.*, 1981; Tsambaos & Orfanos, 1982; Bronson, Fretzin & Farrell, 1983; Berger & Levin, 1984; Metzker *et al.*, 1984; Slifman, Harrist & Rhodes, 1985) (clinical details are listed in Table 1). Seven male and four female infants were found to have congenital asymptomatic patches or plaques on the trunk or upper extremities. Hyperpigmentation and hypertrichosis were prominent features in seven of the cases. However, hyperpigmentation was not observed clinically or histologically in four cases, including the present case, despite the presence of hypertrichosis.

Several authors observed a transient elevation or 'vermicular squirming' (Stokes, 1923) in the lesions after mechanical trauma. The production of this pseudo-Darier's sign has been attributed to the smooth muscle origin of the tumour and serves as a useful aid in the physical

TABLE 1. Clinical characteristics of reported cases of congenital smooth muscle hamartomas

| Author | Sex | Onset at birth | Location | Lesion size and description | Hyper-pigmentation | Hyper-trichosis | Pseudo-Darier's sign |
|---|---|---|---|---|---|---|---|
| Sourreil *et al.*, 1969 | F | + | Lumbosacral | 3 × 4 cm slightly elevated patch | − | + | + |
| Bonafe *et al.*, 1980 | M | + | Left flank | 10 × 10 cm patch | + | + | + |
| Karo and Gange, 1981 | F | + | Anterior abdomen | 3 × 4 cm plaque | + | + | + |
| Chapel *et al.*, 1981 | M | + | Right posterior shoulder | 4·5 × 2·8 cm macule | + | + | NR |
| Tsambaos and Orfanos, 1982 | M | + | left elbow | 10 × 5 cm patch with perifollicular patches | − | − | NR |
| Bronson *et al.*, 1983 | M | + | Lower back | 3 × 4 cm plaque | − | + | NR |
| Berger & Levin, 1984 | F | + | Right upper arm | 7·5 × 5 cm plaque | − | + | NR |
| Metzger *et al.*, 1984 | M | + | Right thigh | 5 × 1·5 cm plaque | + | + | NR |
| | M | + | Back | 3 × 2 cm plaque | + | + | NR |
| | NR | + | Lower back | 4 × 3 cm plaque | + * | + | + |
| Slifman *et al.*, 1985 | M | + | Right posterior thorax | Grouped plaques | + | + | + |
| Present case | F | + | Left flank | 5 × 6 cm plaque | − | + | + |

NR: Not reported; +: present; −: absent; + *: present after one year.

examination. An attempt by Urbanek and Johnson (1978) to reproduce piloerection after intralesional injections of acetylcholine and adrenomimetic drugs in a 13-year-old female with Becker's naevus and smooth muscle hamartoma was unsuccessful.

Follow-up studies by Metzker *et al.* (1984) at 1 and 2 years have shown no change in the size of the lesions, but more hypertrichosis within the lesions compared to the surrounding skin. The change in the degree of skin induration was variable. In our case, there was a marked diminution in skin induration and in the pseudo-Darier's sign at follow-up after 1 year.

No long-term studies have been done to determine the progression of these lesions or to analyse the prognosis of children with congenital smooth muscle hamartomas. Patients with Becker's naevi have developed congenital bony and soft tissue abnormalities (Glinick *et al.*, 1983). To date, congenital smooth muscle hamartomas have shown no evidence of associated congenital abnormalities or malignant transformation.

Congenital smooth muscle hamartoma should be regarded as a distinct clinicohistopathological entity separate from Becker's naevus. The presence of congenital smooth muscle hamartoma at birth, the pseudo-Darier's sign, and the histological findings of dermal smooth muscle proliferation, support this view.

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# Perineural xanthoma

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## SUMMARY

A 50-year-old man suffering from cholestatic hepatitis and diabetes mellitus with hyperlipoproteinaemia had small, painful, slightly elevated, reddish, firm indurated plaques on his soles. Histologically, the lesions were composed of a centrally located cutaneous nerve surrounded by concentric layers of xanthoma cells. Electron microscopy showed the cutaneous nerves to be unmyelinated, their axons were vacuolated and contained dense bodies. The xanthoma cells had the same ultrastructural features as those observed in usual xanthomatous lesions. We suggest that this entity be named perineural xanthoma.

There are several clinical types of xanthoma associated with hyperlipoproteinaemia. They often occur secondarily in patients with diabetes mellitus and obstructive liver disease. Although histologically an accumulation of foam cells is characteristic of all clinical types of xanthoma, their mode of accumulation is not related to the different clinical types.

We describe a type of xanthoma of peculiar clinical and histological appearance, and with a perineural location. To our knowledge, no reports of similar cases have been published.

## CASE REPORT

A 50-year-old man was first seen in November 1982, because of small painful plaques on his soles. He had had cholestatic hepatitis and diabetes mellitus since June 1981, and was being treated with prednisolone and insulin.

Cutaneous examination showed firm, oval, reddish, slightly elevated, indurated plaques 5 × 10 mm on his right sole (Fig. 1) and 6 × 6 mm on his left sole. These plaques were extremely tender and were diagnosed clinically as acute panniculitis or adiposis dolorosa. There were round, dome-shaped, glistening, firm, slightly yellow papules 8 × 8 mm on the inner aspect of his right knee, 4 × 4 mm on the outer aspect of his left ankle, and 3 × 3 mm on the dorsal aspect of his left foot. These lesions were non-tender, and were diagnosed clinically as xanthoma tuberosum.

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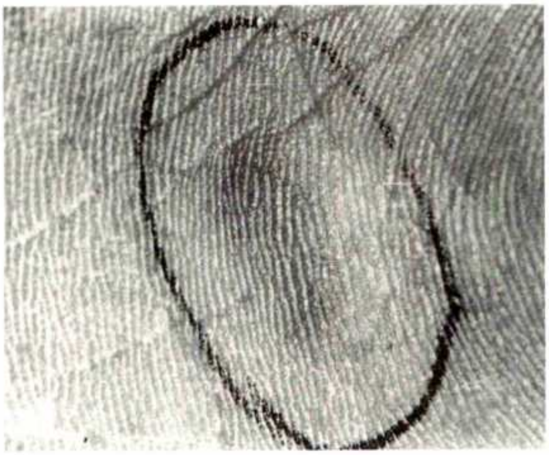

FIGURE 1. A firm, oval, reddish, slightly elevated and indurated plaque on the right sole.

The major abnormal laboratory findings were serum cholesterol 1040 mg/dl, triglycerides 228 mg/dl, total bilirubin 16·4 mg/dl, GOT 78 U/l, GPT 106 U/l, LAP 233 U/l and $\gamma$-GTP 800 U/l

Biopsy specimens were taken from a plaque on the right sole and a papule on the right knee joint. Part of the former was prepared for transmission electron microscopy.

*Light microscopy*

Light microscopy of the plaque on the right sole showed that the epidermis was intact. There were multiple discrete nodular lesions composed of large clear cells and cutaneous nerves throughout the dermis (Fig. 2a). The nuclei of most of the clear cells were small, round or oval and located centrally. Their cytoplasm was highly vacuolated and slightly eosinophilic. Most strikingly, the clear cells were arranged concentrically around the cutaneous nerves (Fig. 2b). Sudan III, Sudan-Black-B and oil red O stains revealed granules of lipids in the cytoplasm of the clear cells, which were thus identified as xanthoma cells. The dermal vessels were slightly dilated and eccrine glands were normal.

The papule on the right knee joint consisted of numerous xanthoma cells in the connective tissue throughout the upper and middle dermis. These xanthoma cells had pale, swollen cytoplasm with small, centrally located nuclei. Mingled with the xanthoma cells were mature collagen fibres and small blood vessels.

*Electron microscopy*

Ultrastructural study confirmed the light microscopic observations. The pattern was of xanthoma cells arranged concentrically around the unmyelinated nerves. These unmyelinated nerves, i.e. axon-Schwann cell complexes, were surrounded by dense endoneural collagen fibres. The intercellular space between the endoneural collagen fibres and the xanthoma cells was filled with a dense amorphous matrix. The endoneural collagen fibres were enclosed by a layer of fibroblastic cells (Fig. 3). The axons were covered entirely by the cytomembrane of the Schwann cells. Some axons were vacuolated and contained dense bodies (Fig. 4a and b). The xanthoma cells contained numerous intracytoplasmic lipid vacuoles without a limiting membrane. Between the lipid vacuoles, myelin figures, mitochondria, rough endoplasmic reticulum and lysosomes of various sizes were found (Fig. 5).

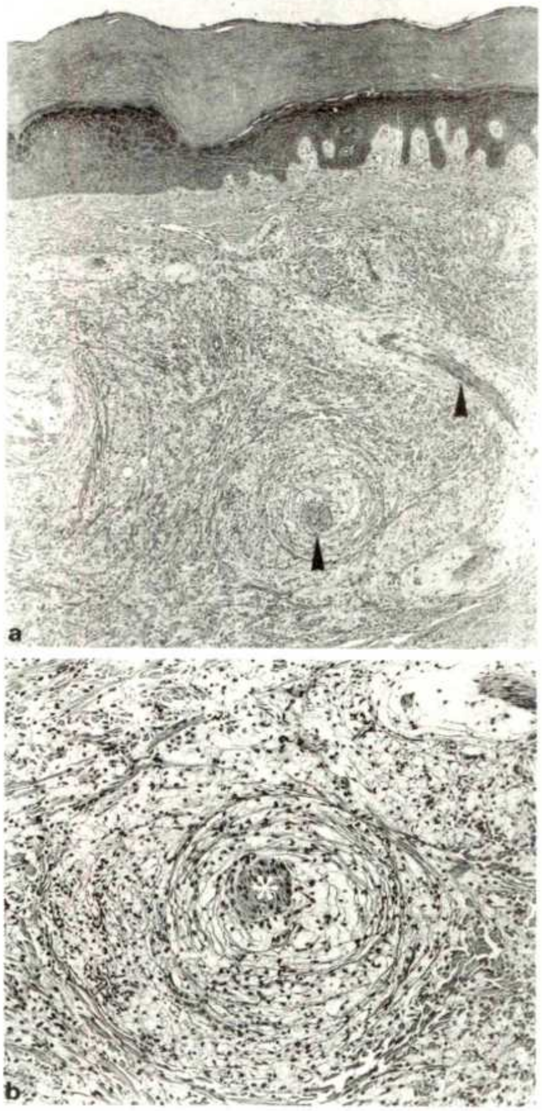

FIGURE 2. (a) Histology of a plaque on the sole reveals almost intact epidermis and dermal lesions composed of cutaneous nerves (arrowheads) and xanthoma cells. (Original × 50). (b) Higher magnification of (a). A cutaneous nerve (asterisk) is situated in the centre of several concentric layers of xanthoma cells.

## DISCUSSION

The patient had long-standing cholestatic hepatitis and diabetes mellitus associated with secondary hyperlipoproteinaemia. Since it is well known that a variety of xanthomas can develop in these disorders, the asymptomatic yellowish papules on the right knee and other parts of the skin were diagnosed clinically as xanthoma tuberosum. In contrast, the lesions on the soles

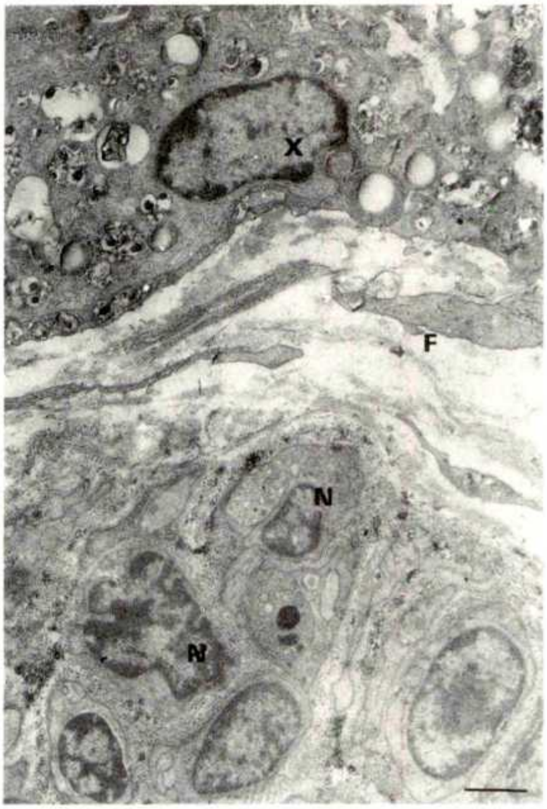

FIGURE 3.Electronmicrograph of plaque on the sole. Xanthoma cells (X) are arranged concentrically around the unmyelinated nerves (N). There are layers of fibroblastic cells (F), and amorphous dense material and endoneural collagen fibres in the intercellular space between the cutaneous nerve and the xanthoma cells. (Original × 4800; scale bar = 2 $\mu$m).

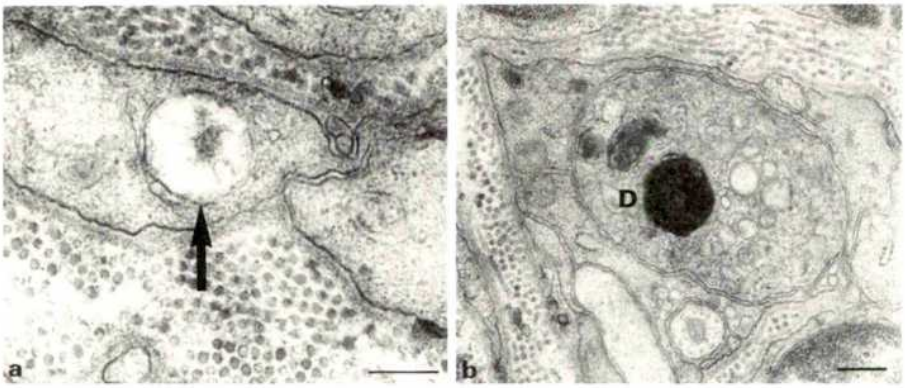

FIGURE 4. Unmyelinated nerve fibres, axon-Schwann cell complexes in the endoneural collagen fibres. (a) Axons are vacuolated (arrow). (Original × 28 000; scale bar = 0·4 $\mu$m). (b) Axons contain dense bodies (D). (Original × 18 000; scale bar = 0·4 $\mu$m).

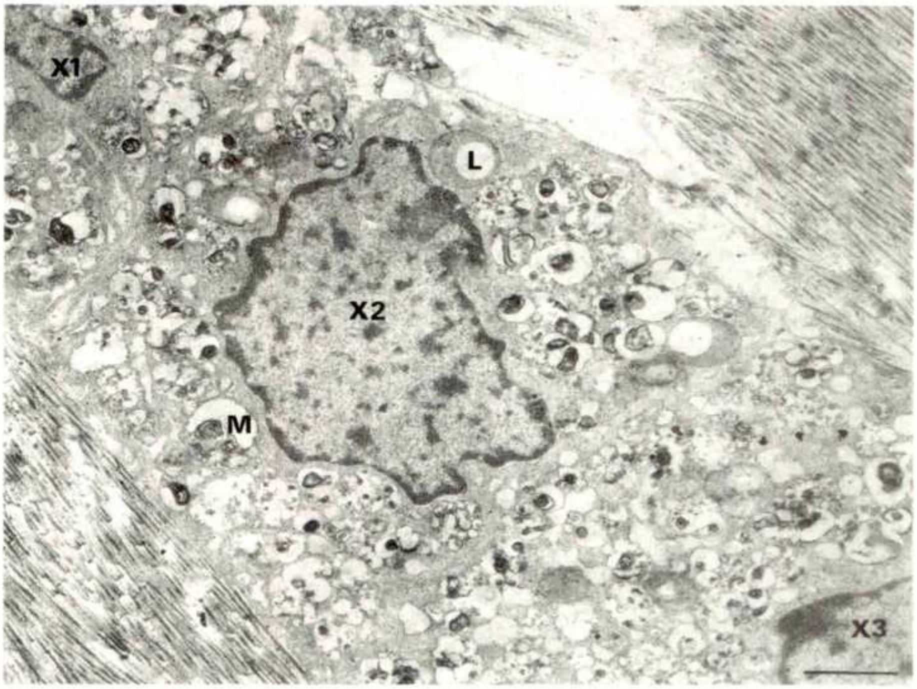

FIGURE 5. The nuclei of xanthoma cells ($X_1, X_2, X_3$) are polygonal with marginated heterochromatin. The cytoplasm is occupied by numerous lipid vacuoles (L) of various sizes. Between the lipid vacuoles are numerous myelin figures (M) of varying sizes. (Original × 7400; scale bar = 2 $\mu$m).

were small, painful, reddish, slightly elevated plaques which were severely tender. The clinical appearance was suggestive of acute panniculitis, adiposis dolorosa or related conditions. Histologically, the papule on the knee showed the typical features of xanthoma. The plaque on the sole was composed of cutaneous nerves and xanthoma cells; a unique histological pattern was formed by the two elements, with a cutaneous nerve situated in the centre forming a core, and several layers of xanthoma cells surrounding it concentrically. These units were scattered throughout the dermis. Such a concentric arrangement of xanthoma cells has been seen around blood vessels (Parker & Odland, 1968; Ferrando & Bombi, 1979) and hair follicles (Pirozzi & Gross, 1971). It would seem, therefore, that xanthoma cells have a tendency to accumulate in a concentric fashion around blood vessels, hair follicles and cutaneous nerves. However, as the lesions on the knee did not show this distinctive histological pattern, it is possible that it may be formed only on the sole.

Another unique feature of this case was that the patient had complained of pain in the lesions on the soles. It is unusual for xanthoma to cause pain. Electronmicroscopically, the Schwann cells showed no pathological changes, but the axons were vacuolated or contained dense bodies. These degenerative changes in the axons were probably caused by diabetes mellitus, not by xanthoma, and these axonal changes may be the cause of the pain and tenderness.

The lesions on the soles in this patient represented a clinically and histologically very unusual type of xanthoma. To our knowledge, this form of xanthoma has not been reported previously, and we suggest that it should be named perineural xanthoma.

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# Release of prostaglandin $D_2$ and histamine in a case of localized heat urticaria, and effect of treatments

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## SUMMARY

A case of localized heat urticaria in a 70-year-old woman is reported. Increased plasma levels of prostaglandin $D_2$ and blood histamine after heat challenge indicate a role for mast cell degranulation in the pathophysiology of the syndrome. Treatment with astemizole increased the temperature threshold to wealing, but not to itch or erythema. The patient was partially desensitized by repeated exposure to heat and this was further improved by indomethacin. After treatment there was no increase in plasma prostaglandin $D_2$ on challenge. No evidence was found for the activation of the alternative complement pathway.

Localized heat urticaria is a rare type of acquired physical urticaria characterized by wealing and erythema limited to the site of heat challenge. Fewer than two dozen cases have been reported worldwide and the patient we describe here is only the third case to be reported in the U.K. (Greaves *et al.*, 1974; Tatnall, Gaylarde & Sarkany, 1984). This form of heat urticaria can be differentiated from the more common cholinergic urticaria by the local response of the skin to heating, although systemic symptoms can occur if the heat-challenged area is sufficiently large (Delorme, 1969).

The mechanism of localized heat urticaria is not known and the responses to treatment variable. Histamine has been found after heat challenge in perfusates of wealed skin (Greaves *et al.*, 1974) and in plasma (Atkins & Zweiman, 1981; Grant *et al.*, 1981; Irwin *et al.*, 1985; Tatnall *et al.*, 1984), implying a role for mast cell degranulation in the pathophysiology of the disease. However, in another study, mast cells were found to remain intact and histamine was not

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released (Daman *et al.*, 1978). It has also been suggested that activation of the alternative complement pathway is involved (Daman *et al.*, 1978; Johansson *et al.*, 1984).

Mast cells contain several preformed inflammatory mediators besides histamine and on degranulation can synthesize others. Prostaglandin $D_2$ ($PGD_2$) is the major product derived from arachidonic acid when isolated lung and peritoneal mast cells are stimulated to degranulate (Lewis *et al.*, 1982). Although its function in immediate type reactions is not clearly understood, $PGD_2$ is known to promote inflammation (Flower, Harvey & Kingston, 1976; Soter *et al.*, 1983). We report here the results of investigations on a patient with localized heat urticaria, including an assay for plasma levels of $PGD_2$, before and after heat challenge and the clinical response after inhibition of $PGD_2$ synthesis.

## CASE REPORT

A 70-year-old retired female school teacher presented with a 10-year history of recurrent redness, itching, burning and swelling of the skin localized to areas exposed to increased temperature. The wealing eruption was induced regularly by bathing or washing in hot water, by leaning on hot radiators, by hairdryers and by direct or indirect exposure to warm sunlight. The patient had noted swelling of the oral mucosal surfaces upon drinking or eating hot foods. Symptoms started within 5 min of exposure and usually lasted from 30 to 60 min. Systemic symptoms of dizziness, headache, sweating and nausea often occurred after extensive exposure. Although the patient tried to avoid triggering stimuli, she remained substantially affected by the urticaria, which was becoming progressively worse.

The patient did not develop urticarial reactions to over-heating, exercise or emotional excitement. Other than recurrent facial erysipelas, endogenous leg eczema and mild hypertension, she was well. She had no history of atopy. Medications included Navidrex K, Naseptin and 1% hydrocortisone cream.

### *Laboratory investigations*

Tests for TPHA and VDRL were negative. Full blood count, ESR, protein and immunoelectrophoresis were normal. Serum immunoglobulins were within the normal range: IgE 115 ng/ml; IgG 8·1 g/l; IgA 2·3 g/l and IgM 1·0 g/l. The C1 esterase inhibitor level was normal; C3 and C4 levels, measured by single radial diffusion, were 40 and 50% of normal adult values respectively, but the level of factor B was normal. The levels of C3, C4 and factor B in blood draining the forearm did not change during heat challenge either before or after treatment of the patient. Immune complexes, antinuclear factor and cryoglobulins were not detected. Tests for pyroglobulins were negative.

### *Clinical investigations*

The clinical diagnosis of localized heat urticaria was confirmed by experimental induction of localized weals. A water-filled glass beaker (6·5 cm diameter) kept at constant temperature was applied to the skin of the thigh or abdomen for 1 or 4 min. The water temperature was increased at each exposure until symptoms of itch and burning were reported by the patient. Clinical signs of erythema and wealing were assessed by an independent observer. Using this method, the wealing threshold at 4 min was 43°C and at 1 min 47°C. Itching and erythema were evoked at slightly lower temperatures. On exposure at 44°C for 4 min, itch began at 1 min and became severe with associated burning by 5 min after application and lasted for 40 min. Erythema was first observed when the beaker was removed at 4 min and persisted for between 60 and 90 min.

Wealing began at 7 min, became confluent at 15 min and lasted for 60 min. The clinical course of lesions induced at 47°C for 1 min was similar except that the onset of wealing was earlier at 4 min. No erythematous flare outside the urticated area was seen.

Other physical urticarias were eliminated by appropriate testing. Induced reactions were not blocked by pre-treatment with 6% aqueous hyoscine hydrochloride, although it blocked local sweating. Monochromator light tests from 300 to 600 nm showed no wealing response, but irradiation of the skin of the thigh by a xenon arc solar simulator induced a typical wealing reaction. If the skin was fan cooled to prevent concomitant heating during irradiation then there was no urticarial response. This indicated that the sun-induced wealing was heat and not UV or visible light induced and eliminated a diagnosis of solar urticaria.

Serial 3 mm punch biopsies of thigh skin were taken before and at 5, 15 and 90 min and 24 h after challenge for 4 min with a beaker containing water at 44°C. The biopsy specimens were fixed in formol saline, embedded in paraffin and stained with haematoxylin and eosin. Skin structure was normal at 5 min and at later times changes were minimal. At 15 min, there was slight oedema with a few perivascular eosinophils, which were also seen at 24 h. At 90 min, there was some endothelial swelling and slight vascular dilatation, and a mild perivascular lymphocyte infiltrate. Polymorphonuclear cells were not seen at any time. Normal numbers of mast cells were seen and degranulation was not evident in chloracetate esterase-stained sections. Immunofluorescence studies at 15 min on frozen sections of urticated skin showed no evidence of C3, IgG, IgA, IgM or fibrin.

## METHODS

### *Materials*

Bond Elut extraction columns were obtained from Jones Chromotography, Glamorgan, Wales, U.K.; $PGD_2$ from Sigma Chemical Company, Poole, Dorset, U.K.; $[^3H]PGD_2$ from Amersham International, Aylesbury, Bucks, U.K.; Pico-Fluor 30 was from Packard, Caversham, Berks, U.K. The $PGD_2$-methyloximate antiserum was produced at the MRC Reproductive Biology Unit, Edinburgh, U.K.

### *Sample collection*

A 19 g (1·1 mm internal diameter) butterfly cannula was inserted into the antecubital vein of the right forearm and kept patent with a slow infusion of normal saline. The arm was challenged by immersion to the elbow in water at 44·5°C for 5 min. Blood samples (20 ml) were collected for $PGD_2$ analysis before, during and after heat challenge and mixed with 300 units lithium heparin in cooled tubes. The samples were centrifuged at 1400 *g* for 10 min at 4°C and the plasma removed and stored at −70°C until analysed. Separate 1 ml blood samples were collected for bioassay of histamine.

### *$PGD_2$ radioimmunoassay*

A 1 ml aliquot of plasma was diluted with an equal volume of ethanol:water (1:4 v:v) and acidified with 0·01 ml acetic acid. The mixture was allowed to equilibrate for 5 min and was then centrifuged at 10 000 *g* in an Eppendorf microcentrifuge for 5 min. The supernatant was applied to a 3 ml C18 Bond Elut extraction column which had been previously equilibrated with ethanol and then ethanol:water (1:9 v:v). The extraction column was then washed with 5 ml water followed by 3 ml hexane. $PGD_2$ was eluted with 1·5 ml ethyl acetate. The ethyl acetate in the sample was removed by evaporation under a stream of nitrogen.

The $PGD_2$ in the lipid extract was measured by radioimmunoassay (RIA) using a rabbit antiserum specific to $PGD_2$-11-methyloximate (Kelly *et al.*, 1986). Briefly, the plasma lipid extract was redissolved in 0·1 ml of methyloximating solution (450 ml 1 M sodium acetate, 5 g methoxyamine hydrochloride and ethanol to a final volume of 500 ml, pH 5·7) and then diluted with an equal volume of assay buffer (0·1 M tris-HCl buffer, pH 7·4, containing 0·336 g/l EDTA, 0·5 g/l sodium azide and 5 g/l bovine γ-globulin). Samples were heated to 60°C for 30 min to give $PGD_2$-methyloximate and diluted with a further 0·2 ml assay buffer before RIA. Each RIA assay tube contained 0·1 ml of serially diluted sample or standard $PGD_2$-methyloximate, 7000 dpm $[^3H]PGD_2$-methyloximate in 0·1 ml RIA buffer and 0·1 ml of a 1:16000 dilution of antiserum to $PGD_2$-methyloximate. The tubes were equilibrated at 4°C overnight and the bound tracer precipitated with 0·8 ml 25% (w/v) polyethylene glycol and centrifuged at 1400 ***g*** for 30 min. The supernatant was aspirated and the pellet resuspended in 1·2 ml Pico-Fluor 30 and the radioactivity determined by liquid scintillation counting.

Unlabelled standard $PGD_2$ and tracer $[^3H]PGD_2$ required for the RIA were methyloximated by a similar procedure. Cross reactivity of the antiserum to $PGE_2$, $PGF_{2\alpha}$ and 22 other major prostaglandins or metabolites was less than 0·3%. The 50% displacement of $[^3H]PGD_2$-methyloximate binding was given by 8 pg $PGD_2$-methyloximate and the linear range was 3–30 pg $PGD_2$-methyloximate.

### *Histamine assay*

Blood histamine levels were measured by the cascade superfusion bioassay using two guinea-pig ilea in sequence (Lawrence *et al.*, 1981), with histamine activity on the second ileum antagonized by mepyramine.

### *Release of inflammatory mediators on heat challenge*

The release of $PGD_2$ and histamine into the venous blood draining the right forearm was measured after heat challenge before and after treatment of the patient. A female subject aged 59 years and with no known clinical disorder acted as a control. Immersion of the right forearm in water at 44·5°C for 5 min was the challenge in each case.

## RESULTS

Results for $PGD_2$ are shown in Table 1. In the patient before treatment the plasma $PGD_2$ concentration 8 min from start of heat challenge was 23 times higher than the pre-challenge value. $PGD_2$ levels then declined but were still elevated at 32 min. The control subject had no detectable increase in plasma $PGD_2$ on heat challenge. Recovery of $PGD_2$ from the extraction columns was approximately 90%; the values shown have not been corrected for the small losses which occurred during purification.

Blood histamine was below the limit of detection of the bioassay (<2·5 ng/ml) in the untreated patient before heat challenge and in the control subject at all times. In the patient, following heat challenge, the blood histamine level increased in parallel with $PGD_2$ to a maximum of 12·5 ng/ml at 8 min (Table 1).

The clinical response in the control subject was a slight tingling of the fingers at 1 min and a pale pink erythema when the arm was removed from the warm water. The erythema had faded by 17 min. In contrast, the patient reported that the arm and hand were painful at 5 min when removed from the water and there was erythema. At 7 min, there were small individual weals and itching. By 10 min, the weals were coalescing and the dorsum of the hand was swollen. At 20

TABLE 1. Plasma PGD$_2$ levels before and at intervals after initiating heat challenge for 5 min, measured in the patient before and after treatment, and in a control subject not sensitive to heat. Blood histamine levels in the patient before treatment are also given

| | Blood histamine (ng/ml) | Plasma PGD$_2$ (pg/ml) | | |
|---|---|---|---|---|
| Time after start of challenge (min) | Patient | Patient | | Control |
| | pre-treatment | pre-treatment | post-treatment | |
| Pre-challenge | <2·5 | 18 | 31 | 24 |
| 4 | 6 | 12 | ND | 21 |
| 8 | 12·5 | 420 | 30 | 17 |
| 12 | 11 | 285 | 48 | 16 |
| 16 | 10 | 175 | 30 | 19 |
| 32 | 7 | 87 | ND | 10 |

ND = not determined.

min, the erythema was fading and the wealing had decreased, but swelling was still present. Erythema was still present at 40 min but the swelling over the dorsum of the hand had diminished. Itching was restricted to the palms.

### *Treatment*

The patient was given three forms of treatment. The first approach was to suppress the effects of released histamine by means of an $H_1$ inhibitor. Astemizole, 10 mg/day for 6 days, increased the wealing threshold from 44 to 47°C, using the beaker test, but did not alter the threshold for erythema or itch. Increasing the dose to 30 mg/day for 3 more days increased the threshold for wealing, erythema and itch to above 48°C. Although asymptomatic, the patient was unable to continue the therapy because of profound sedation.

The second approach was to desensitize the patient, a method reported to have been effective in two other cases (Leigh & Ramsay, 1975; Tatnall *et al.*, 1984). Initial tests demonstrated localized heat tolerance at supra-threshold and slightly less at sub-threshold temperatures after repeated hot water beaker exposures at 90 min intervals. Eleven baths, each of 4 min duration, were given daily with the water temperature starting sub-threshold at 39°C and increasing 0·5°C every alternate bath to a final temperature of 41·5°C. Each day more of the body was exposed until on day 4 the entire body except the head was immersed and the number of baths taken was reduced to six. On subsequent days, the starting bath temperature was slowly increased and the number of baths taken reduced until on day 11 two baths at 41·5°C were taken 8 h apart. For the next 5 days, two baths at 41°C were taken 10 min apart. There was no wealing, only slight itch on the lower legs and no systemic effects during the 3-week course of desensitization. After desensitization, the threshold had increased to 48°C in a 4 min beaker test. The patient had a slower response, less itch and wealing and was able to take baths at a higher temperature, but she still developed wealing when washing-up and with hot sun exposure.

Because desensitization was not entirely satisfactory, and because of the demonstration before treatment of raised plasma PGD$_2$ levels after heat challenge, indomethacin 25 mg four times a day was added to the tolerance programme. The temperature threshold in the beaker test was not raised further but there was a small clinical improvement. Induced urticaria only occurred focally on the left hand and on the face in warm sunlight. A repeat challenge test (immersion of the forearm in water at 44·5°C for 5 min) did not cause raised plasma $PGD_2$ levels (Table 1). Histamine was not detected in the blood. The clinical response was milder than in the pre-treatment test. There was itching and erythema when the arm was removed from the water and at 7 min there was some oedema of the flexor aspect of the wrist. Wealing did not become confluent as in the initial test. At 10 min, there was irritation of the arm. Wealing began to fade by 15 min and erythema by 30 min.

## DISCUSSION

The cause of localized heat urticaria is unknown and because the condition is extremely rare no single investigator has been able to study a group of affected patients. Localized heat urticaria is differentiated from cholinergic type heat urticaria in which there is a generalized papular response to a rise in body temperature due to exercise, overheating or emotional excitement. Approximately 75% of patients with localized heat urticaria are female. Only rarely do they suffer from other forms of physical urticaria, but up to 60% have been reported to have a history of atopy or elevated IgE levels.

In our patient, we have demonstrated for the first time in localized heat urticaria the generation of $PGD_2$, an arachidonic acid metabolite which can be considered a specific indicator of mast cell activation (Holgate *et al.*, 1984). The time course of $PGD_2$ release into the plasma was approximately the same as for histamine and coincided with the appearance of the clinical signs and symptoms after challenge. Lower baseline levels of $PGD_2$ in plasma have been reported by Barrow *et al.* (1984) using a gas chromatography–mass spectrometry (GCMS) assay. The direct RIA we used is probably less specific than the more rigorous GCMS assay, but this does not invalidate our results.

Our results confirm earlier studies showing the release of histamine into the blood (Atkins & Zweiman, 1981; Grant *et al.*, 1981; Irwin *et al.*, 1985; Tatnall *et al.*, 1984), and in one instance in perfused skin (Greaves *et al.*, 1974). The activation of mast cells leading to the release of preformed inflammatory mediators and the concomitant generation of newly formed mediators is clearly implicated in the pathogenesis of localized heat urticaria in several cases, although the specific nature of the stimulus which activates the mast cells is not yet known.

Two reports have shown a decrease in factor B levels in plasma after heat challenge, implying activation of the alternative complement pathway (Daman *et al.*, 1978; Johansson *et al.*, 1984), but in several other cases no changes in complement or complement activator levels were found (Atkins & Zweiman, 1981; Grant *et al.*, 1981; Starr, Brasher, Hammerschmidt, 1980; Tatnall *et al.*, 1984). We found low levels of C3 and C4 before challenge which suggests a persistent activation of the classical complement pathway, but there was no change on heat challenge, implying that this abnormality is unlikely to be related to the patient's urticaria. The normal factor B levels, also unchanged on heat challenge, indicate no activation of the alternative complement pathway. Local complement activation in the skin was not demonstrated.

Histological studies revealed some endothelial swelling and tissue oedema and a sparse mononuclear cell infiltrate which is in general agreement with the results of Johansson *et al.* (1984). Electron microscopic studies by Daman *et al.* (1978) showed endothelial disruption with extravasation of leukocytes and erythrocytes. However, their patient differed slightly from others reported in that the clinical response was slightly delayed in onset, and was non-pruritic, tender and indurated. A high challenge temperature, 56°C, was used and some of the histological changes could have been non-specific effects of high temperature alone. Although we were able to demonstrate the release of mast cell mediators, histology of the challenged skin did not show evidence of mast cell degranulation. It is possible that only a small proportion of the mast cells was degranulated. However, electron microscopic studies would have given clearer evidence for the extent of mast cell degranulation.

Treatment of localized heat urticaria remains difficult and avoidance is impractical. Few patients have responded to antihistamines, and although our patient did respond to high doses of antihistamines, they were not of value because of side-effects. Irwin *et al.* (1985) recently reported that in two subjects, $H_1$ and $H_2$ antagonists given alone were ineffective, but combined therapy abolished the clinical responses. This does not exclude the possibility that other mediators derived from mast cells are important in the pathophysiology of localized heat urticaria. Their subjects differed from our patient in having a 10°C higher threshold temperature for a clinical response, and the challenge temperature used to demonstrate a release of histamine was 56°C. The effects of this severe heat challenge on control subjects without localized heat urticaria was not studied. Desensitization by the induction of physical tolerance has been reported to be successful in two cases (Leigh & Ramsay, 1975; Tatnall *et al.*, 1984). In our patient, even though local tolerance was demonstrated, desensitization was only partially effective. Addition of indomethacin to the desensitization treatment resulted in a small further improvement, and with the combined treatment there was complete suppression of $PGD_2$ release to the blood.

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# Oesophageal lichen planus

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## SUMMARY

A 50-year-old woman with severe oral lichen planus complained of painful dysphagia. Fiberoptic endoscopy revealed erosive lichen planus of the oesophagus which responded to treatment with systemic corticosteroids.

Lichen planus (LP) not uncommonly affects mucous membranes of the mouth and pharynx. Whilst involvement of the genitalia, anus and gastric mucosa have been well documented (Scully & Et-Kom, 1985), there have been very few reports of oesophageal involvement.

## CASE REPORT

A 50-year-old woman gave a one-month history of a sore mouth. For 6 years she had suffered from chronic ulcerative vulvovaginitis. No firm diagnosis had been made; biopsy had shown chronic inflammation only. Examination of the buccal cavity showed extensive erosive LP of the tongue, lips, gums and buccal mucosa. She was prescribed local hydrocortisone. Subsequently, she developed increasingly severe, painful dysphagia located at the level of the upper sternum. There was no past history of heartburn or other symptoms of oesophageal reflux.

Fiberoptic endoscopy revealed erosive changes in the oesophagus extending to 25 cm from the incisors. There were areas of desquamation, the separated mucosa hanging off in sheets. In other areas, white arborizations were seen. Contact bleeding was readily induced. The lower oesophagus, stomach and duodenum were normal. Biopsies from the affected upper oesophagus revealed non-specific inflammatory changes with basal cell hyperplasia and lymphocytic infiltration.

A diagnosis of oesophageal LP was made and treatment was started with oral prednisolone 20 mg/day, plus cimetidine 400 mg twice a day. Rapid symptomatic improvement followed within days. After 4 weeks, cimetidine was stopped. Symptoms did not recur, but later when her dose of prednisolone had been reduced in stages to 5 mg/day, dysphagia returned. Symptoms resolved rapidly when prednisolone dosage was increased to 20 mg/day without cimetidine being added. Thereafter, repeated attempts to withdraw corticosteroids resulted in recurrence of dysphagia which always responded quickly within days of increasing the dose. Endoscopy

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was repeated whilst the patient was symptom-free taking prednisolone 10 mg/day; on this occasion no inflammatory changes were seen. She remains maintained symptom-free on a small dose of oral corticosteroid.

## DISCUSSION

Oral involvement is common in lichen planus, occurring in up to 60% of patients (Scully & Et-Kom, 1985). Erosive oral LP is uncommon, occurring in 6% of patients with oral lesions. Reports of oesophageal LP are rare.

Guedon *et al.* (1982) described a patient with a mid-oesophageal stricture initially thought to be peptic in aetiology. Following development of erosive oral LP, the diagnosis was revised to oesophageal LP. Improvement followed treatment with systemic corticosteroids but repeated dilatation of the stricture remained necessary. The authors postulated that the formation of LP at the site of presumed peptic oesophagitis was an example of the Koebner phenomenon.

Ottignon *et al.* (1983) described a similar clinical picture. Following the development of oral LP, they considered that the mid-oesophageal stricture could be due to oesophageal involvement with LP and they obtained histological confirmation. They went on to monitor lower oesophageal pH and concluded that no reflux was occurring and that the stricture was unlikely to have a peptic background. The patient's dysphagia responded to corticosteroid drugs.

Two other patients with erosive oesophageal LP have been documented with details of histology included in the reports (Lefer, 1982; Al-Shihabi & Jackson, 1982). In these patients dysphagia was not associated with an oesophageal stricture. One responded to ACTH.

In our patient there was strong clinical evidence for a diagnosis of erosive oesophageal LP without stricture formation. Histology of biopsies was compatible with, but not diagnostic of LP; this is not uncommonly the case (Shklar & McCarthy, 1961). There was no evidence in our patient of gastro-oesophageal reflux and the oesophagus distal to the lesion was normal endoscopically. It is likely that the lesion was not associated with peptic oesophagitis but was primary lichen planus of the oesophagus. Good clinical and endoscopic evidence suggests responsiveness to systemic corticosteroid drugs as would be expected on the basis of previous reports.

Oesophageal involvement with erosive LP is rare but diagnosis is important. Differentiation from peptic oesophagitis will allow successful treatment with corticosteroids, which are ineffective and contra-indicated when symptoms are caused by acid or bile reflux.

The development of squamous cell carcinoma is a rare complication of oral LP. It is more likely to occur when there has been prolonged erosive LP. The possibility of malignant change suggests that those rare patients with symptomatic erosive oesophageal LP should receive vigilant follow-up.

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# Recurrent angioedema: familial and oestrogen-induced

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## SUMMARY

We report two sisters who both have had severe attacks of urticaria and angioedema only when taking the contraceptive pill and during the latter half of pregnancy. Although they exhibited many features in common with hereditary angioedema (HAO), the C1 esterase inhibitor levels and other complement components were consistently normal, including levels measured during pregnancy and at the height of the eruption after oestrogen challenge. Aetiological factors in relation to other patterns and causes of urticaria are discussed.

## CASE REPORTS

### *Case 1*

This patient, a 29-year-old housewife, first developed urticaria in 1974 at the age of 18, when she was started on Ovulen 50 (ethinyl oestradiol 50 $\mu$g). During the first, second and third menstrual cycles she developed attacks of angioedema affecting the hands, feet, face and throat, starting between the 15th and 20th day of each cycle and lasting 4–5 days. Once the Ovulen was stopped, no further trouble occurred. On restarting a different contraceptive pill a year later, she again developed angioedema which ceased on stopping this pill.

In 1979, at the age of 24, she became pregnant, and at about 19 weeks the angioedema returned, becoming more severe in the last 2 months of pregnancy. The swellings usually lasted 12–18 h and often occurred at sites of mild pressure or blows, developing half an hour after the injury. On one occasion, she cut her finger-tip on a can and 2 h later a swelling developed which gradually spread up the arm as far as the elbow over a period of 18 h. Sometimes, colicky pain accompanied by nausea preceeded the onset of swelling by 24 h. A male infant was born in June 1980. After delivery, the patient had swellings around the mouth at the site of the anaesthetic mask and also on the vulva. Within 1–2 days, she was free of angioedema and remained so until her second pregnancy.

In 1981, she again became pregnant and at about the 16th week the swellings reappeared; these were more frequent and severe than during the first pregnancy. The mouth and throat were often affected, at times causing difficulty in breathing. A female infant was born in October 1981 and one day later the swellings remitted and she was again completely clear.

During this second pregnancy she was admitted to Southmead Hospital on a number of occasions, and at the time various treatments were tried, including fresh frozen plasma, intravenous prednisolone and amino-caproic acid. There was no benefit except from amino-caproic acid, which ameliorated her symptoms a little.

She was seen in 1982 as she wanted to have a further pregnancy. A diagnosis was made of recurrent angioedema, familial and hormone-precipitated. Test doses of progesterone were administered; Provera (medroxyprogesterone acetate) 5 mg daily was given for 21 days, starting at day 5 of the cycle. There was no effect. Ethyloestradiol 30 μg daily was then started, beginning on day 4 of the cycle. Seven days later, a swelling developed on the forearm lasting 24 h, and 2 days after this she had abdominal pain and nausea followed by swellings on the left foot, right hand and face. She continued with the same dose for 21 days and by then the recurrent swellings were so severe that she was admitted to hospital as an emergency. At this time, her whole face, fauces and uvula were swollen and she had difficulty in swallowing. Widespread weals were also present over the trunk and limbs. The condition cleared completely 1–2 days after stopping the oestrogen.

She has since been followed up for 2 years and has had no further problems. A prick test to oestrogen 1 mg/ml was negative. The two children, now aged 5 and 3, are quite healthy.

Her general health has always been good. She had hay fever from April to August each year and was found to have positive prick tests to pollens, cat fur, dog hair and house dust mite.

### *Laboratory tests*

Blood taken during the patient's second pregnancy and just after an attack of angioedema showed normal functional activity of both the classical and alternative pathways of complement and C1 esterase inhibitor. Immunochemical measurements of C3, C4 and C1 esterase inhibitor also produced normal results at this time, and when she was well and not pregnant, immediately before challenge tests with hormones, after progesterone, and at the height of an attack of angioedema after oestrogen. Other investigations including full haematological studies and autoantibody tests were normal throughout. A mildly elevated serum IgE (150 IU/l, normal less than 122 IU/l); was evident on one occasion.

### *Case 2*

This patient, a 32-year-old housewife and the sister of Case 1, had had no relevant skin problems until the age of 20 when, having been on a contraceptive pill which contained 50 μg ethinyl oestradiol (Ovulen 50) for 12 months, at monthly intervals she developed extensive angioedema, particularly affecting the face and limbs. The lesions tended to occur at the mid-point of the cycle and lasted for 24–96 h. She therefore stopped the pill and the swellings disappeared. After a 2-year break, she took a 30 μg oestrogen-containing pill (Microgynon 30) and her symptoms recurred during the second cycle. She therefore discontinued this pill and the swellings disappeared until she became pregnant 4 years later.

During this pregnancy, she developed similar swellings and weals in the 13th week. The worst affected sites were the hands, arms, feet and face, and the swellings lasted for up to 72 h. The angioedema worsened in the 26th week, necessitating admission to hospital, and hospitalization was required on four other occasions. During these severe episodes, she had troublesome oedema of the throat resulting in dyspnoea and dysphagia. Although the swelling usually developed for no obvious reason, trauma such as injections could induce oedema 30 min later, and the patient considered that mental stress also aggravated the problem. The swellings and mucosal oedema ceased 48 h after delivery. She did not breast feed.

There were no more problems until the second pregnancy 4 years later, when she developed swellings on the feet and hands in the 13th week. These episodes continued intermittently throughout the pregnancy, but were less than during the first pregnancy. She only required two admissions to hospital, both for throat swellings, which, however, were not as severe as in the previous pregnancy. The symptoms again cleared 2 days after delivery and she did not breast feed. On both occasions, the baby was a male; the second child had hypospadias, but the first child had no problems. Antihistamines of many types had no effect, nor did amino-caproic acid. Oral steroids were not given. Adrenalin spray helped the mouth swelling, but that was only used a few hours after the delivery of the first child.

### *Laboratory results*

Blood samples taken in the 16th and 24th week of her first pregnancy and at weekly intervals for 6 weeks prior to her second pregnancy always showed a normal profile for the functional measurements of the classical and alternative complement pathways and immunological measurements of Clq, C3, C4, C1 esterase inhibitor and factor B.

### *Family history*

There is one brother, aged 24, and he, their mother, father and the rest of the family have had no angioedema or relevant rashes.

## DISCUSSION

There are a number of associations between urticaria and sex hormones. Perhaps the best known is the effect of progesterone. A few cases have been described, notably by Farah and Shbaklu (1971) in which a sensitivity to progesterone appeared to be the precipitating factor. Zondek and Bromberg (1945) drew attention to the possibility of an allergic sensitization to endogenous hormones, including oestrogen. Three out of 13 patients had chronic urticaria but the evidence of oestrogen sensitivity was not clear cut and, as far as we are aware, there have been no cases of urticaria due to oestrogens reported since.

Another association is the marked increase in weals and swellings of idiopathic chronic urticaria which quite commonly occurs in the premenstrual phase (Warin & Champion, 1974). It has often been thought that this was a secondary effect of premenstrual tension but the changes in fluid balance and in blood vessels at such a time are complex and might well have an effect on an underlying urticarial tendency. One of us (R.P.W.) has recently treated five such patients with progesterone during these few days before the onset of menstruation. This prevented exacerbations of the urticaria, but pre-menstrual tension was also less, leaving the mechanism in doubt.

In our two patients, the dramatic effect of oestrogens occurred with both synthetic oestrogen and also presumably the increased endogenous oestrogen in pregnancy. This suggests mechanisms other than an allergic response.

The history of swellings and abdominal colic occurring in two sisters is strongly suggestive of hereditary angioedema (HAO). However, this is ruled out by the investigations, particularly the functional C1 esterase inhibitor and C4 estimations, which at all times, including at the height of the swellings, were within normal limits.

In this connection, a patient seen recently by one of us (R.P.W.) is interesting. She was a married woman, aged 32, with a typical HAO and with C1 esterase inhibitor 0·05 g/l (normal range 0·15–0·33 g/l), rising to 0·10 g/l on treatment with stanozolol. Very slight swellings began

17 years ago. She started to take a contraceptive pill (type unknown) 2 years later, and the swellings became severe and continued for 4 months until she discontinued the pill. After a few years, she started a contraceptive pill again and 2–3 days later she had severe swellings which settled down when she stopped the pill. She has three children, aged 10, 6 and 2 (all with serological changes characteristic of HAO) and during each pregnancy, swellings were worse, although she did not require admission to hospital.

Gordon *et al.* (1980) demonstrated a slight decrease in plasma C1 esterase inhibitor in normal females on contraceptive pills. They also mention a patient aged 17 with HAO who had barely detectable functional C1 esterase inhibitor and had her first significant episode of cutaneous oedema 3 weeks after starting a contraceptive pill.

Apart from HAO, other types of urticaria sometimes have a familial incidence. This may occur with idiopathic chronic urticaria and some families with this condition also all show a marked worsening of the urticaria after salicylates. Similarly, recurrent idiopathic angioedema without, or with minimal, urticarial weals may have a familial incidence. Occasionally, even the physical urticarias may be familial, and this applies particularly to symptomatic dermographism and to cold urticaria. In all these patterns of urticaria no abnormality of complement has been demonstrated. However, in view of the clear-cut enzyme inhibitor deficiency which explains HAO, and has been known for over a decade, it would seem likely that in the future other inhibitory enzyme deficiencies may be demonstrated and explain these familial cases.

In the absence of further information, we can only speculate as to the mechanism of the action of oestrogen in our patients. Sex hormone-related substances can act on various enzyme systems and, for example, in HAO there is a dramatic effect of stanozolol and danazol on the level of C1 esterase inhibitor. However, the clinical condition of these two patients is so similar to HAO that it may be that there is some similar but unidentified enzyme abnormality and that this is affected by oestrogens.

## ACKNOWLEDGMENTS

We would like to thank Dr J.Whicher of Bristol, Professor G.Gowland of Leeds, Dr A.M.Ward of Sheffield and Dr M.Heaney of Salford for serological investigations.

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# Acropustulosis of infancy

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## SUMMARY

Acropustulosis of infancy is a syndrome characterized by recurrent pruritic acral vesicopustules. It occurs primarily in black male infants and hitherto has been described mainly in the North American literature. We describe four cases seen in London. This entity, therefore, should be recognized in the U.K., particularly in the differential diagnosis of infantile scabies.

Cases of acropustulosis of infancy (AI) were presented sporadically at meetings in North America in the 1970s. In 1979, 12 cases of AI were described in the *Archives of Dermatology* (Jarratt & Ramsdell, 1979; Kahn & Rywlin, 1979), and Jennings and Burrows (1983) reported a further four cases. There has been only a single report in the British literature of this condition (Verbov & Morley, 1983). We describe here four typical cases seen in London.

## CASE REPORTS

### *Case 1*

A male infant of West Indian parents was said to have had tiny papules on his toes and fingers at birth. These resolved spontaneously within 1 day. Similar lesions recurred at 3 months of age on the dorsa of the hands, on the finger webs (Fig. 1) and on the sides and dorsa of the feet. These lesions were intensely pruritic, leading to loss of sleep, and occurred in crops at intervals of 7–10 days. Individual lesions evolved from a 1–2 mm papule through a vesicular phase and left a brown macule. The child was otherwise well, although there was some clinical evidence of mild atopic eczema. The lesions had been unresponsive to treatment with scabicides and topical steroids.

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Case 1 was presented to the Section of Dermatology, Royal Society of Medicine, in March 1985.

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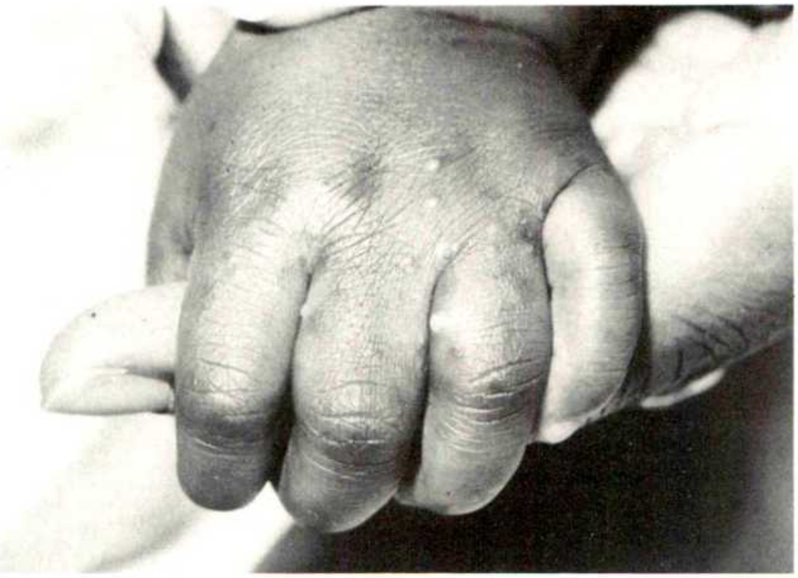

FIGURE 1. Case 1. Typical vesico-pustules on the dorsum of the hand and on the finger webs.

*Investigations.* Swabs taken for bacteriology and virology were sterile, skin scrapings for *Sarcoptes scabei* were negative and haematology was normal.

New lesions continued to erupt for a further 6 months. Pruritus was partially relieved by increasing the dose of oral antihistamines. At 8 months of age, spontaneous remission occurred.

### *Case 2*

A male infant of West Indian parents presented at the age of 14 months with a history of an itchy vesico-pustular eruption of acral distribution present from birth. Crops of vesico-pustules occurred every few weeks and each took 2 or 3 weeks to resolve. The areas affected were predominantly acral, although occasional lesions had been noted on the frontal scalp and extensor aspects of the knees. His general health remained good throughout. There had been no response to treatment with topical hydrocortisone.

Examination revealed groups of vesico-pustules 1–2 mm in diameter on the hands, feet and ankles. There was hyperpigmentation and scaling of the adjacent skin. There was no clinical evidence of scabies.

*Investigations.* Swabs for bacteriological examinations were sterile and skin scrapings for scabies were negative.

The eruption was continuing at 17 months of age.

### *Case 3*

A 9-month-old baby of an Asian family developed itchy vesicles on the borders of his feet. The diagnosis of eczema was made initially but the lesions were unresponsive to topical steroids and antibiotics. The eruption, at presentation to us 1 year later, was characteristic of AI with 2 mm pustules and papules limited in distribution to the lateral borders of the feet.

TABLE 1. Characteristics of reported cases of acropustulosis of infancy

| Authors | No. of cases | Ethnic origin | | | | Sex ratio M:F | Age at onset (months) | Age at resolution (months) | Dermatopathology | Additional features |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Black | Malay | Asian | White | | | | | |
| Jarratt and Ramsdell (1979) | 10 | 9 | — | — | 1 | 9:1 | 2–10 | 24 plus | Neutrophil pustules | — |
| Kahn and Rywlin (1979) | 2 | 2 | — | — | — | 1:1 | Birth | 25–26 | Neutrophil pustules | — |
| Lucky and McGuire (1982) | 1 | — | — | — | 1 | Male | Birth | 17 | Eosinophil pustules | Atopic eczema |
| Jennings and Burrows (1983) | 4 | 2 | 1 | — | 1 | 3.1 | Birth–8 months | 6–24 | Neutrophil pustules | — |
| McFadden and Falk (1985) | 2 | — | — | 1 | 1 | 1:1 | 12–17 | Unknown | Neutrophil pustules | One case atopic<br>One case high IgE |

The condition persisted for 2 years and as a result of an increase in severity sulphapyridine was subsequently started at 0·5 g daily. Rapid resolution of the lesions followed, but the AI relapsed 3 days after the sulphapyridine was stopped. The condition again settled when sulphapyridine was re-introduced. A second flare also responded to sulphapyridine, convincingly demonstrating the value of this drug in this case of AI.

Spontaneous resolution of the AI finally occurred at 3½ years of age.

### *Case 4*

A male of Asian parentage presented at the age of 21 months with a 12-month history of itchy papules and pustules on the borders and dorsa of his hands and feet. He had received antiscabetic treatment 1 month prior to presentation with no resultant improvement in his symptoms.

Examination revealed the typical lesions of AI with erythematous papules and pustules particularly marked on the lateral borders of the hands and feet. There were no burrows. General examination revealed only mild eczematous changes at the nape of the neck and on the legs. The eruption was unresponsive to topical steroids and therefore after 15 months of disease activity sulphapyridine was started at 0·5 g daily. After 1 month, partial resolution had occurred.

## DISCUSSION

Acropustulosis of infancy is a disease predominantly of black male infants. Intensely pruritic acral papulo-vesicles develop early in infancy with an onset from birth up until 10 months of age, and with spontaneous resolution at 6–48 months (Jarratt & Ramsdell, 1979; Jennings & Burrows, 1983; Kahn & Rywlin, 1979; Lucky & McGuire, 1982) (Table 1). The lesions occur in crops lasting 1–2 weeks (Jennings & Burrows, 1983). No organisms can be detected in the skin and the histology typically shows intra-epidermal or subcorneal neutrophilic pustules, although the infiltrate may contain eosinophils (Lucky & McGuire, 1982). Direct immunofluorescence is negative (Kahn & Rywlin, 1979; McFadden & Falk, 1985).

Although AI is a self-limiting condition, the pruritus is distressing and the therapeutic response to antihistamines and topical steroids is frequently poor (Jarratt & Ramsdell, 1979; Kahn & Rywlin, 1979). Kahn and Rywlin (1979) reported a striking response of two patients to sulphones. Similarly, we demonstrated the efficacy of sulphapyridine in our Case 3; sulphapyridine was selected as this is better tolerated in children (Grant, 1968). However, Case 1 was controlled adequately with improved antihistamine cover, and as AI is essentially transient, sulphones or sulphonamides should probably be restricted to use in particularly intractable cases. Sulphapyridine specifically is no longer marketed in the U.K., but is available direct from manufacturing company, May and Baker Ltd (Dagenham, U.K.).

AI bears a marked clinical similarity to infantile scabies, and thus many of the reported cases have received scabicides, although the mites were not demonstrated, with a predictably poor therapeutic response. Bjornberg and Friis (1978) described 32 Asian infants adopted into Swedish families. All had AI clinically, and 13 of the 32 had had positive scrapings for scabies prior to the onset of the AI. Elpern (1984) described seven patients with AI, four of whom had had antecedent scabies. It is therefore possible that in a percentage of infants with AI, the disease process may represent an abnormal reaction, in the predisposed individual, to the scabies infestation. However, it is difficult to explain the onset of the AI at birth (as seen in two of our cases) in this way.

AI should be considered in the differential diagnosis of infantile scabies.

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# *Obituary*

## Claude Howard Whittle

Claude Howard Whittle, who died recently in Cambridge, was born at Felixstowe on 2nd May 1896. His father was a schoolmaster and died when Howard was still a young child.

Howard was educated at the Masonic School, Bushey, Hertfordshire. He won a scholarship to Queens' College, Cambridge in 1916. Before he qualified he went to France with the Friends Ambulance Unit and in 1921 he became MRCS and LRCP. He spent six months each as House Surgeon, House Physician and Casualty Officer, and one year as Resident Clinical Pathologist at Kings College Hospital.

In 1923 he returned to Cambridge where he remained for the rest of his life. He worked first as Assistant Cinical Pathologist to Dr J.Gaskell in the John Bonnett Laboratory at Addenbrookes. Dr Gaskell was known for his extreme conservatism. He refused to own a car or typewriter and refused to carry out widely used investigations, including the Wassermann reaction.

Dr Whittle became MRCP in 1924 and Honorary Assistant Physician in charge of the Skin Department, in which appointment he succeeded Dr John Aldren Wright, a general practitioner in Histon. Wright had established a department in 1908 which had been used primarily for the follow-up after X-ray epilation of school children in school medical services paid for by local authorities and which gradually became a general dermatological clinic. Clinical pathology was a rapidly developing speciality which had not yet defined its boundaries. In many hospitals, including Addenbrookes, the department was responsible for blood transfusions.

In 1948, when Dr Gaskell retired, the pathology services were reorganized and Howard began to confine his work to dermatology. He played a large part in the development of dermatology at Cambridge and in the clinics he opened at Peterborough, Wisbech, Bury St Edmunds, Bedford and Papworth Village Settlement. During the war years he was the only dermatologist in the Eastern counties.

He attended regularly the meetings in London of the Royal Society of Medicine and was Secretary of the Dermatology Section from 1937 to 1949. He was Secretary of the British Association of Dermatologists when Sir Humphrey Rolleston was President in 1937 and was himself President in 1953–54.

Within dermatology his particular interest was mycology, on which he published a number of papers, including fundamental and frequently quoted work on the pathogenesis of chronic paronychia. Dr Whittle was a past President of the British Society of Mycopathology and was a familiar and much loved figure at the Society's annual meetings which he regularly attended until two years prior to his death.

He was greatly interested in the Arts. He painted, mainly in water colours, and was President of the Cambridge Drawing Society. He was also an enthusiastic small boat sailor and was President of the Sailing Club for many years.

A.J.R.

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## Lichen sclerosus et atrophicus in non-identical female twins

MADAM, We were interested to read the report by Drs Meyrick-Thomas and Kennedy (*British Journal of Dermatology*, 1986, **114**, 377) describing the development of perineal lichen sclerosus et atrophicus in a pair of monozygotic female twins. We have recently seen the same disorder in a pair of non-identical female twins, who developed lesions at the age of 2 years and $2\frac{1}{2}$ years respectively. Both girls are otherwise well; their sister (aged 5 years) is unaffected, and there is no family history of autoimmune disease. Their mother had had granuloma annulare several years previously.

The striking feature of these twins, like those described by Meyrick-Thomas and Kennedy, was the development of lesions in both twins within a 6-month period. This supports the feeling that there may be a genetic aspect in some cases of lichen sclerosus et atrophicus.

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## Gamma-glutamyl transpeptidase as a marker for distinguishing malignant epithelial tumours

MADAM, Drs Chiba and Jimbow (*British Journal of Dermatology*, 1986, **114**, 459) have suggested the use of gamma-glutamyl transpeptidase (GGT) as a marker for distinguishing malignant epithelial tumours. They have inferred that absence of GGT from basal cell epithelioma (BCE) supports the view that it is not a carcinoma.

In an earlier paper we reported greatly increased GGT activity in psoriatic plaque (Hajini & Hussain, 1980). As such, GGT cannot be considered a specific marker for malignancy and its absence does not support the view that BCE is not a carcinoma.

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### REFERENCE

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## Influence of containers on the quantities of topical preparations used

MADAM, Many hospital pharmacies have been forced by budget limits to restrict the dispensing of topical agents. We wished to discover if smaller packs of cream change the rate of use, and also to compare the rate of use when the cream is dispensed in tubes in place of pots.

Twenty-one healthy adult female volunteers took part in the study, forming three matched groups of seven. Each volunteer was given Aqueous Cream B.P. to apply to the hands and forearms twice daily for

TABLE 1. Use of Aqueous Cream related to size and type of container

| Container | No. of subjects | Amount of cream used (g) | |
|---|---|---|---|
| | | Mean ± SD | Range |
| 100 g pot | 7 | 26·5 ± 12·5 | 5·5–46·0 |
| 100 g tube | 7 | 33·2 ± 13·7 | 6·0–48·5 |
| 200 g pot | 7 | 64·5 ± 21·4 | 37·5–106·0 |

12 days (25 applications). The cream was dispensed in three types of container: a 100 g pot, a 100 g tube and a 200 g pot. The exact quantity used was determined by weighing before and after the study, and the results are shown in Table 1.

The quantities of cream used (mean 1·1–2·6 g per application) compare with the amount for a single whole arm application (1·8–1·9 g) reported previously (Schlagel & Sanbourn, 1964). Our results show that about twice as much cream was used from the larger pot compared with either of the smaller containers. Use from the larger pot was 143% more than from the smaller pot. There was significantly more cream used when the cream was dispensed in a tube compared with a pot of similar size ($P < 0{\cdot}001$; Student's *t*-test). This is possibly due to the difficulty of returning excess to the container.

Our results apply to healthy skin. There are certain practical limits to varying the amount of topical agent applied and extrapolation would be misleading. While these results would seem to support dispensing smaller quantities as an economy measure, no account is taken of any consequent reduction in effectiveness.

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### REFERENCE

SCHLAGEL, C.A. & SANBORN, E.C. (1964) The weights of topical preparations required for total and partial body inunction. *Journal of Investigative Dermatology*, **42**, 253.

## Lasers, dermal fibroblasts and psoriasis

MADAM, Drs Békássy and Astedt recently reported (*British Journal of Dermatology*, 1986, **114**, 489) long-term remissions of circumscribed fixed psoriatic plaques after carbon dioxide laser vaporization. Remission of circumscribed psoriasis has also been reported after superficial skin shaving (Kill, Kill & Søgaard, 1985) and after argon laser irradiation or cryotherapy (Harrison, Walker & Davies, 1985).

These treatments are fundamentally similar. Each destroys not only the epidermis but also the upper dermis. The lasers destroyed the lesions to 'a depth of approximately 1 mm'. The dermatomes were set to remove epidermis and dermis 'to the level of the mid reticular dermis'. The long-term remissions obtained with these controversial therapies have pathophysiological implications and emphasize the role of the dermis in psoriasis.

*In vivo*, psoriatic plaques are characterized by hyperproliferative keratinocytes. However, the rates of proliferation of normal and psoriatic keratinocytes do not differ in monoculture (Liu & Parsons, 1983).

Thus, extra-epidermal factors are implicated. When full-thickness psoriatic skin is grafted onto the nude mouse, firstly the epidermis from lesions maintains most of its pathological features for at least 6 weeks and, secondly, the uninvolved epidermis develops certain features characteristic of the lesion (Fraki, Briggaman & Lazarus, 1983). The primary defect in psoriatic skin, therefore, may originate within the skin itself.

Since living dermal elements are needed to facilitate and direct growth and differentiation of normal epithelia (Mackenzie & Fusenig, 1983), the role of dermal fibroblasts in inducing plaque formation has been investigated *in vitro*. Dermal fibroblasts from either normal or psoriatic skin, previously grown as conventional monolayer cultures, were combined with normal epidermal cells in a 'living skin equivalent' model (Saiag *et al.*, 1985). Psoriatic fibroblasts, but not normal fibroblasts, induced hyperproliferation in normal keratinocytes. Thus, the primary defect in psoriatic skin may lie in the dermal fibroblasts.

The long-term remissions obtained after destroying the superficial dermis provide *in vivo* support for the pathophysiological role of dermal fibroblasts in psoriasis. In addition, it provides a basis for speculation that keratinocyte hyperproliferation in psoriasis is not induced by all dermal fibroblasts, but only by the upper dermal ones, those which are selectively destroyed by these therapies. Thus, dermal fibroblasts may not all be identical but may be 'specialized' according to their anatomical location, as recently demonstrated for dermal papilla fibroblasts (Jahoba, Horne & Oliver, 1984). Indeed, the fibroblast cultures used by Saiag *et al.* (1985) originated from superficial skin biopsies.

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## Cyclosporin A treatment of severe psoriasis

MADAM, I was interested in the report of the effectiveness of cyclosporin A (CyA) in psoriasis (*British Journal of Dermatology*, 1986, **114**, 615) because of an open trial I am carrying out with this drug in patients with long-standing, severe psoriasis. As yet, only one patient has completed the full 24 weeks of the trial, but preliminary results suggest that the response is good and occurs well before this. The regimen is to give CyA 1 mg/kg/day, increasing the dose by 1 mg/kg/day every 2 weeks if response does not occur, up to a maximum dose of 5 mg/kg/day.

Clearance of psoriatic lesions has occurred after 2–12 weeks (mean 7·4 weeks) with doses of between 1 and 5 mg/kg/day (mean 3·3 mg/kg/day). Serum levels of CyA (mean 54·3, range <20–137 ng/ml) have been well below those usually regarded as toxic. No adverse effects, particularly on blood pressure and renal function, were noted in any of the patients.

Details of the eight patients who have followed the trial regimen, so far for 6–24 weeks, are shown in

Table 1. All but one have cleared (completely clear or less than 5% skin surface involved) and the patient who has not was the only one who had generalized pustular, and not chronic plaque, psoriasis. Two patients withdrew from the trial, one because of nausea and the other because of exacerbation of hiatus hernia symptoms due to stopping ranitidine when she entered the trial.

While accepting that these results are preliminary and that the trial is an open one, there is no doubt that CyA can clear psoriasis. Smaller doses than those previously described (van Joost *et al.*, 1986; Mueller & Herrmann, 1979; Harper, Keat & Staughton, 1984; J. Voorhees, personal communication) are effective, and it may be safer to use small doses of CyA long term than the usually accepted drugs like methotrexate. Thus, CyA may have a place in maintaining clearance of psoriasis and not only in 'crisis intervention' as suggested in the recent article. Larger doses may be needed for such intervention and failure to respond to the low-dose regimen would not rule out such a possibility. Reports agree that relapse occurs quickly when CyA is stopped and this was certainly true of Patient 6, whose psoriasis recurred to its original extent within 2 weeks of stopping the drug. Improvement is occurring with trough serum levels (measured by radioimmunoassay) lower than those used to prevent graft rejection, so the drug's mode of action in psoriasis may not be the usual immunological one. In the small group of patients studied there was no obvious relationship between response and trough serum CyA levels, and this parameter may not be the only one relevant in these patients.

TABLE 1. Patient details and outcome of CyA treatment

| Patient | Age (years) | Duration of psoriasis (years) | Previous treatments which failed | Weeks of trial to date | Weeks to clearance | Dose of CyA at clearance mg/kg/day | Trough serum level of CyA at clearance ng/ml |
|---|---|---|---|---|---|---|---|
| 1 | 33 | 26 | MTX, PUVA, ET | 18 | 2 | 1 | < 20 |
| 2 | 36 | 31 | MTX, PUVA | 6 | 6 | 3 | 46 |
| 3 | 66 | 20 | MTX, PUVA, ET, OH-urea | 6 | 6 | 3 | ND |
| 4 | 64 | 27 | MTX, PUVA, ET, Azathioprine | 12 | 10 | 3 | 137 |
| 5 | 57 | 12 | — | 10 | 10 | 3 | < 20 |
| 6 | 40 | 23 | MTX, PUVA, ET | 24 | 6 | 5 | 50 |
| 7 | 50 | 47 | MTX, PUVA, ET, OH-urea | 12 | 12 | 5 | 53 |
| 8 | 65 | 35 | MTX, PUVA, OH-urea, Azathioprine | 23 | Not clear | 5* | 46* |

MTX = methotrexate; ET = etretinate; ND = not determined.
* At 23 weeks.

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*British Journal of Dermatology* (1986) **115**, 747–749.

# Book Reviews

**Mercurialis on Diseases of the Skin. The 16th Century Physician and his Methods. The First Book on the Subject (1572).** Translation from the Latin, Glossary and Commentary by RICHARD L. SUTTON, JR. (1986) Kansas City: Lowell Press. Pp. 226.

Two heroes of Dermatology here! Mercurialis (1530–1606), who wrote his textbook of dermatology during the reign of Elizabeth I, and Richard L.Sutton, Jr., who has translated it from the Latin for us neo-Elizabethans. In 1572, Mercurialis, who for long held the Chair of Medicine at Padua, published in Venice his *De Morbis Cutaneis*. Dr Sutton of the celebrated Sutton dermatological dynasty (Sutton's naevi, etc.), has given us a magnificent volume in a stiff Oxford blue cloth cover imprinted in gold with a medallion of Hieronymus Mercurialis. The translation is scholarship in unstodgy English prose. Introductory chapters and the extended, laconic, 80-page glossary, 'Identifications', place Mercurialis and medicine within the historical confines of the sixteenth century, and can be enjoyed independently of the main text.

Book One of *De Morbis Cutaneis* concerns diseases of the head and hair. Of the 11 chapters, those entitled Pediculosis, Tinea, Pityriasis and Sycosis have a modern ring. The six chapters in Book Two are introduced by the first, a brief explanation of classification introducing 'diseases of the skin of the whole body'. 'On Pruritus' contains gems '... pruritus a kind of pain, and, as Plato said ... a pleasurable sensation from scratching, and, Socrates, as he scratched his shackled legs, said ...': one-third of Mercurialis's 70-odd references are from BC authors! and they include, of course, Hippocrates. Scabies, (the scabby disease) 'is closely related to pruritus' but plainly encompasses manifold conditions, and Mercurialis has sensible advice on hygiene and a warning against the danger of poisoning your patient with mercury.

Aetiology is founded upon the basic faith in the Four Humours and the concept that the skin is an excretory organ (a figment still current among patients and in rare circumstances with histopathologists!). Mercurialis agrees with Avicenna that sexual agitation excites vapours harmful to the skin. And, as one reads, one encounters the quaint and interesting. Perhaps one's bent is to try and sort out what diseases he is talking about. 'On Lepra', eczema and psoriasis, I would guess, but the Greek and the Arab and the Jewish types are all most confusing, as indeed Mercurials himself admits, and, 'On Lichens', well, maybe even Galen throws in the sponge.

Our home-grown Daniel Turner, the first English dermatologist, publishing his own *De Morbis Cutaneis* (1714) in the vernacular, quotes Mercurialis, and deals with disease of the scalp and hair separately from that of the skin of the body. Turner is a gorgeous disorderly jumble, but the later William, and thus Bateman, follow the Mercurialis classification more closely. Like William Heberden, who observed the skin closely (describing succinctly Henoch-Schönlein purpura three-quarters of a century before Schönlein), Mercurialis had much of his written material published posthumously; Mercurialis by his students, Heberden by his son (1802) who translated it from his father's Latin.

Publishing professional mysteries in the language of the common people of the country was a betrayal of union rules. The colourful Carthusian, Andrew Boord (1490–1549), scholar-physician to Henry VIII, wrote several dermatological chapters in English and met professional trouble. But our profession now is scarcely 'learned' and the humanities are neglected, so Dr Sutton doing the same sort of thing centuries later has enriched medical writing by presenting fascinating and important Mercurialis to the un-latined.

P.W.M.COPEMAN

**Dermatological Surgery.** Edited by J.L.VERBOV. Lancaster: M.T.P. Press. Pp. 140. Price £25.00.

This is the first volume in a planned series of a new publication on dematological topics under the editorship of Dr J.Verbov. This short book on dermatological surgery is easy to read, not only because the print is of an easily readable size, but also because the contents are presented in a simple, straightforward way. The book begins with a chapter on dermatological surgery by Dr J.S.Comaish of Newcastle and this forms a very useful introduction to the volume. Lasers in dermatology are covered very adequately by Mr J.S.Carruth, a veteran therapist in this field, whilst the applications of cyrosurgery are well documented by Dr R.P.R.Dawber.

I think any jobbing dermatologist would find the data presented in these three chapters both useful and stimulating. However, this reviewer found the final chapter on cosmetic surgery less relevant to his own practice, although a plastic surgeon could be pleased by its contents. The volume will be useful not only to dermatologists in training, but also to those who regard themselves as trained. On the whole, the series editor has done his job well, but it is inevitable that in a multi-authorship volume there will be some differences in opinion. For instance, one author is for the use of phenol to induce facial peeling, and another is against it.

The cost of this volume is £25.00 and there may be some, and not only those living in the northern parts of these islands, who feel that this is a little excessive for a slim volume of 140 pages.

J.A.Cotterill

**Recent Advances in Sexually Transmitted Diseases.** Edited by J.D.Oriel and J.R.W.Harris (1986) Edinburgh: Churchill Livingstone. Pp. 277. Price £35.00.

Five years after the last volume, the editors present 16 chapters as a partial account of the expanding frontiers of genito-urinary medicine.

Three chapters deal with genital HPV infections, two with gonorrhoea and two with AIDS, while there is one each on infections related to *Haemophilus ducreyii*, herpes simplex, hepatitis B and chlamydiae. The others are of a less specific nature, and survey genital ulceration in the tropics, bacterial vaginosis, reactive arthritis and Reiter's syndrome, proctocolitis in homosexual men, and the provision of services for sexually transmitted diseases in developing countries.

Perhaps as the price paid for the quick production needed to let such a book be up-to-date, there are scattered errors in the way of missing words and references as well as spelling mistakes. Some chapters are a little crammed and cramped, probably as a result of the enthusiasm of the authors. However such blemishes do not detract from the overall value of the work.

Herpes simplex, HPV and AIDS (all subjects very well covered) are of obvious relevance to the dermatologist. Of the other contributions I would particularly commend the chapters on reactive arthritis and Reiter's syndrome, and on proctocolitis in homosexual men, as helpful reviews of matters which impinge on the dermatologist's day-to-day work, and on which he may need authoritative guidance.

C.M.Ridley

**Essential Allergy. An Illustrated Text for Students and Specialists.** Niels Mygind (1986) Oxford/ Boston: Blackwell Scientific Publications. Pp. 480. Price £17.50.

This is an acceptable and useful book, particularly for clinicians. At first glance it appears superficial, but more attentive reading shows that it lucidly describes the essential features of antibodies, mediators and antibody-mediated allergies. The book is essentially an account of clinical aspects of allergic reactions associated with IgE, with several chapters on asthma. Skin diseases described are atopic dermatitis, urticaria and hereditary angioedema. However, the relatively short account of cutaneous allergy should not deter dermatologists from reading the introductory chapters on mechanisms. The text is clearly and attractively presented with many useful illustrations. There are few references, the chapters being concluded with a few brief abstracts of relevant papers.

W.E.Parish

**The Human Skin** (The Institute of Biology's Studies in Biology no. 164).
E.J.WOOD AND P.T.BLADON (1985) London: Edward Arnold. Pp. 66. Price £3.95.

The authors of this compact book have baulked at nothing in filling it to overflowing with up-to-date information. They discuss inflammation and prostaglandins, list immunoglobulins, tabulate the structures of the triple helix in the different types of collagen and even venture into the contentious field of cutaneous sensation. The material is, however, oddly assembled. An introductory chapter has sections on glands, innervation, blood supply and skin colour, all of which is repeated elsewhere, and a piece on skin flora and fauna, including lice and the scabies mite. It is succeeded by an account of the epidermis which includes stratified epithelium, lipids, sebaceous glands and hair—including diagrams of hair distribution on the male body and the development of pattern baldness, nails and dermatoglyphs. A similar treatment of the dermis includes a section on the sweat glands! A model chapter on melanogenesis deals logically with melanin formation, ultraviolet radiation and skin cancer. The final chapter is a pocket guide to skin disorders which incorporates further material on sebaceous secretion, epidermal kinetics and enzymes concerned in collagen formation.

F.J.G.EBLING

## Books Received

**The Clinical Use of Electron Microscopy in Dermatology.** A.S.ZELICKSON (1985) Minneapolis: Bolger Publications. Pp. 79.

**The Nails in Disease.** P.D.SAMMAN and D.A.FENTON (1986) 4th edition. London: Heinemann. Pp. 215, Price £25.00.

**Adams and Maegraith: Tropical Medicine for Nurses.** B. MAEGRAITH and H.M.GILLES. 5th edition. Oxford: Blackwell Scientific Publications. Pp. 366. Price £19.50.

*British Journal of Dermatology* (1986) **115**, 750.

# *News and Notices*

## British Society for Dermatopathology

The next meeting of the Society will be held in the Nevin Lecture Theatre of St Thomas's Hospital, London, on Friday, 27 February 1987. The theme of the meeting is The Role of Dermatopathology in the Diagnosis of Internal Disease. Topics to be discussed include cutaneous manifestations of paraprotein production, mechanisms in vasculitis, histiocytotic syndromes, tuberculosis and the tuberculides, and metabolically active tumours, and there will be a slide seminar. Further details are available from Dr P.H.McKee, Department of Histopathology, St Thomas's Hospital Medical School, Lambeth Palace Road, London SE1 7EH.

## Institute of Dermatology—New Drugs for Treatment of Skin Diseases

With the generous support of Janssen Pharmaceutical Company, the Institute of Dermatology is holding a two-day course entitled 'New Drugs for Treatment of Skin Diseases' on 3 and 4 September 1987 at the Royal Society of Medicine, 1 Wimpole Street, London W1. Topics to be covered by the invited faculty, which will be internationally constituted, will include interferons, lipocortins, cyclosporins, lipoxygenase inhibitors, calcium channel antagonists, gonadotrophin releasing hormones, minoxidil, antimicrobials and photoprotectives. In addition, there will be a mini-symposium on new antihistamines. Enquiries from interested participants should be addressed to the Course Director, Professor M.W.Greaves, or his administrative assistant, Mrs Rosemary Barton, at the Institute of Dermatology, Homerton Grove, London E9 6BX, from whom further details are available.

## European Society for Dermatological Research—Symposium on Ageing and Skin Cancer

The ESDR is holding a clinically oriented symposium on Ageing and Skin Cancer to be held at the Hotel Midas Palace, Rome, Italy from 15 to 17 February 1987. Enquiries should be addressed to the Organizing Secretariat, ESDR Symposium, c/o P.T.S. s.r.l., Viale Bruno Buozzi 61, 00197 Rome, Italy. Telephone 06/80 45 53, Telex: 621514 PTS-ROM I. Scientific committee: F.Serri, G.Rabbiosi, D.Cerimele and A.Giannetti, Foundation for Research in Dermatology, Largo Gemelli, 8-00168 Rome, Italy.

## Fellowship in Vulvar Physiology and Disease

The Dermatology Department, University of California School of Medicine, San Francisco, is offering a one-year fellowship for the study of vulvar physiology and vulvar disease. Candidates with training in dermatology or gynaecology will be considered. For information, please write to: Howard Maibach, M.D., University of California, Dermatology, Box 0989, San Francisco, CA 94143, U.S.A.

# Index

## SUBJECTS

A23187 stimulates arachidonic acid release and cyclic GMP accumulation, 297
Acne and unemployment (Correspondence), 386
Acne in women treated with oral spironolactone, 227
Acne, psoriasis and malignant melanoma not associated, 573
Acrokeratoderma associated with punctate keratosis (Case report), 589
Acrokeratosis, paraneoplastic Basex, 595
Acro-osteolyses and the nail unit (Correspondence), 638
Acropustulosis of infancy, 735
Additives (Poster summary), Suppl. 30, 36
Adnexal carcinoma, microcystic, 101
AIDS and the skin (Summary), Suppl. 30, 16
AIDS in the skin (Summary), 585
Alagille's syndrome (Case report), Suppl. 30, 52
Alcohol and psoriasis (Summary), Suppl. 30, 11
Allografts of cultured keratinocytes (Summary), Suppl. 30, 21
Alopecia with prereticulotic eruption (Case report), Suppl. 30, 71
Amiodarone-induced photosensitivity (Correspondence), 253
Amiodarone-induced photosensitivity (Correspondence reply), 254
Amphoteric surfactant on the skin barrier, Suppl. 31, 136
Amyloidosis, primary (Case report), Suppl. 30, 61
Anetoderma of Jadassohn (Case report), Suppl. 30, 102
Angioedema, recurrent, familial and oestrogen-induced, 731
Angio-leiomyoma (Case report), Suppl. 30, 94
Angiomatosis in polyarteritis nodosa, 611
Angry back a new concept? Suppl. 31, 93
Anthralin inflammation (Correspondence), 251
Anthralin irritancy, Suppl. 31, 80
Antiandrogen assays using quail uropygial gland, Suppl. 31, 33
Antiandrogens percutaneously absorbed, Suppl. 31, 36
Antigens, extracellular, in formaldehyde fixed, paraffin embedded tissue (Poster summary), Suppl. 30, 39
Antiseptics inhibiting wound healing, Suppl. 31, 41
Arachidonic acid metabolism and thalidomide, 677
Arachidonic acid metabolism in psoriasis (Correspondence), 517
Arachidonic acid metabolism in psoriasis (Correspondence reply), 518
Arachidonic acid release and A23187, 297
Arsenicism, non-iatrogenic (Case report), Suppl. 30, 102
Arterial lesions of the lower lip (Correspondence), 254
Arterial lesions of the lower lip (Correspondence reply), 255
Arteriohepatic dysplasia (Alagille's syndrome) (Case report), Suppl. 30, 52
Arteriovenous periungual and subungual tumours, 361
Atopic dermatitis and mite antigen, 485
Atopic dermatitis and scratching, 467
Atopic dermatitis, class II HLA expression (Poster summary), Suppl. 30, 38
Atopic dermatitis, effect of salbutamol (Poster summary), Suppl. 30, 33
Atopic dermatitis, mupirocin topically (Summary), Suppl. 30, 27
Atopic eczema, adverse risk factors (Poster summary), Suppl. 30, 31
Atrophia maculosa varioliformis cutis, 105
Azelaic acid, antimicrobial effect, 551

Back, angry. A new concept? Suppl. 31, 93
Basal cell carcinomas, recurrence rates (Summary), Suppl. 30, 23
Basal cell carcinomas, spread (Summary), Suppl. 30, 23
Bazex paraneoplastic acrokeratosis, 595
Bazex syndrome (Case report), Suppl. 30, 97
Behaviour variables and skin diseases (Summary), 586
Behçet's syndrome and thalidomide, 367
Benoxaprofen and phototoxicity (Correspondence), 515
Benoxaprofen and UVR potentiating reactive oxidant generation, 285
Birt-Hogg-Dubé syndrome and lichen sclerosus (Case report), Suppl. 30, 97
Book Reviews:
  *Acquired Immuno Deficiency Syndrome. Progress in Allergy*. Vol. 37. Edited by E.KLEIN, 642
  *Advances in Dermatology*. Vol. 1. Edited by J.P. CALLEN, 121
  *Cutaneous Photobiology*. W.FRAIN-BELL, 387
  *Dermatological Surgery*. Edited by JULIAN VERBOV, 747
  *Essential Allergy. An Illustrated Text for Students and Specialists*. NIELS MYGIND, 748
  *Essentials of Industrial Dermatology*. W.A.D. GRIFFITHS and D.S.WILKINSON, 519
  *Fundamentals of Skin Biopsy*. JUNE K.ROBINSON, 120
  *Fungal Dimorphism, with Emphasis on Fungi Pathogenic for Humans*. Edited by P.J.SZANISLO, 120
  *Infectious Diseases in the Female Patient. (Clinical Perspectives in Obstetrics & Gynecology)*. Edited by R.P GALASK and B LARSEN, 644
  *Low Dose Methotrexate Therapy in Rheumatic Diseases*. Edited by R.RAU, 643
  *Mercurialis on Diseases of the Skin. The 16th Century Physician and his Methods. The First Book on the Subject (1572)*, 747

    *Optoelectronics in Medicine. Proceedings of the 7th International Congress with the 2nd International Nd: Laser Conference, LASER 85.* Edited by W.WIAD-ELICH and P.KIEFHABER, 641
    *Papillomaviruses.* Ciba Foundation Symposium 120, 519
    *Pathogenesis of Skin Disease.* Edited by B.H.THIERS and R.L.DOBSON, 643
    *Pathology of Unusual Malignant Cutaneous Tumours.* Edited by M.R.WICK, 642
    *Pinkus Guide to Dermatohistopathology.* 4th edn. AMIR MEHREGAN, 519
    *Practical Psoriasis Therapy.* N.J.LOWE, 119
    *Précis de Dermatologie et Vénéréologie* J-H.SAURAT, E.GROSSHANS, P.LAUGIER and J-M.LACHAPELLE, 256
    *Recent Advances in Dermatology, Number 7.* Edited by R.H.CHAMPION, 119
    *Recent Advances in Sexually Transmitted Diseases.* Edited by J.D.ORIEL and J.R.W.HARRIS, 748
    *Skin Problems in the Elderly.* Edited by L.FRY, 641
    *The Human Skin (The Institute of Biology's Studies in Biology no. 164)* E.J.WOOD and P.T.BLADON, 749
    *Therapeutic Photomedicine.* Edited by H.HONIGSMANN and G.STINGL, 256
    *Urticaria.* BEATE M.CZARNETZJI, 387
Brachioradial pruritus, 177
Brauer's syndrome (Case report), Suppl 30, 44
Brazilian pemphigus foliaceus elevated thymosin alpha I levels, 147
Bullous pemphigoid antibody localized (Poster summary), Suppl. 30, 29
Burn scars and blisters, 501

Calmodulin levels and psoriasis (Poster summary), Suppl 30, 30
Candida-endocrinopathy syndrome. Carcinoma of the tongue (Case report), Suppl. 30, 100
Carcinogenesis in skin a multistep system, Suppl. 31, 1
Carcinogenic potential, endpoints in short-term tests, Suppl. 31, 117
Carcinoma, microcystic adnexal, 101
Cat scratch disease (Case report), 592
Cellular metabolism and skin toxicity *in vitro* models, Suppl. 31, 108
Cheilitis, granulomatous (Case report), Suppl. 30, 78
Chlorpromazine phototoxicity (Poster summary), Suppl. 30, 35
Cholinergic dermographism, 371
Cold sensitivity and myelomonocytic leukaemia, 607
Collagen lattice contraction in inherited disorders of connective tissue, 139
Collagenase and TIMP in hypertrophic scar tissue, 409
Connective tissue degraded by collagenase and hyaluronidase, 403
Connective tissue inherited disorders and collagen lattice contraction, 139
Contact dermatitis and the microcomputer (Poster summary), Suppl. 30, 36
Contact dermatitis from an epoxy compound (Poster summary), Suppl. 30, 33
Contact dermatitis from 1-methylquinoxalinium-*p*-toluenesulphonate (Summary), Suppl. 30, 26
Contact dermatitis, HLA antigens and Langerhans cell density, 447
Contact dermatitis, prognostic factors (Summary), Suppl. 30, 25
Contact sensitivity in leg ulcers (Poster summary), Suppl. 30, 41
Contact sensitization models, Suppl. 31, 53
Corticosteroids topically, effect on skin, Suppl. 31, 101
Crohn's disease (Case report), Suppl. 30, 80
Crohn's disease, cutaneous (Case report), Suppl. 30, 79
Cryosurgery and sensory loss (Summary), 585
Culture systems defined, Suppl. 31, 17
Cutaneous nodular chain (Poster summary), Suppl. 30, 32
Cutaneous virilism (Poster summary), Suppl. 30, 40
Cutis marmorata telangiectatica congenita (Case reports), Suppl. 30, 50 and 51
Cyclic GMP accumulation and A23187, 297
Cyclosporin A and psoriasis (Correspondence), 249
Cyclosporin A for severe psoriasis (Correspondence), 745
Cyproterone acetate in hidradenitis suppurativa, 263

Darier's disease (Case report), Suppl. 30, 67
Degos' disease (Correspondence), 249
Dermatitis, contact and irritant, immunopathological and ultrastructural findings, 305
Dermatology referrals in a general hospital, 167
Dermatoscopy (Summary), 585
Dermatotoxicity testing evaluated, Suppl. 31, 136
Dermographism, cholinergic, 371
Detoxification systems in epidermis, Suppl. 31, 9
Discoid lupus erythematosus (Case report), Suppl. 30, 54
DNCB absorption through various types of epidermis, Suppl. 31, 47
DNCB sensitization, effect of area and dose, 663
Dowling-Degos disease (Case report), Suppl. 30, 103
Drug eruption to paracetamol, 357
Drugs topically, risks, Suppl. 31, 63

Eccrine porocarcinoma, three cases, 111
Ectrodactyly, ectodermal dysplasia and cleft lip-palate syndrome (Case report), 587
Ehlers Danlos syndrome type VIII (Poster summary), Suppl. 30, 42
Elastase, a marker of neutrophils in skin infiltrates, 181
Endometriosis, cutaneous (Poster summary), Suppl. 30, 38
Epidermal cell outgrowth and platelet factors, 529
Epidermal cell spreading factors, 13
Epidermal cells secreting plasminogen activator inhibitor, 133
Epidermal detoxification systems, Suppl. 31, 9
Epidermolysis bullosa, diagnosis, 123
Epidermolysis bullosa, diagnosis and epidermal basement membrane defect, 125
Epidermolysis bullosa, dominant dystrophic (Case report), Suppl 30, 86
Epidermolysis bullosa in two siblings (Case report), Suppl. 30, 85
Epidermolysis bullosa, mental and physical handicap (Case reports), Suppl. 30, 84

Epidermolysis bullosa, monoclonal antibodies (Summary), Suppl. 30, 20
Epidermolysis bullosa, recessive dystrophic (Case report), Suppl. 30, 86
Epidermotropic T cells, do they exist in human skin? 389
Epoxy compound in cutting oil causing allergic dermatitis (Poster summary), Suppl. 30, 33
Erosive adenomatosis of the nipple (Case report), 594
Erythema multiforme, persistent (Case report), Suppl. 30, 89
Erythroderma and *Staphylococcus aureus*, 693
Erythrokeratoderma variabilis (Mendes da Costa's syndrome) (Case reports), Suppl. 30, 67 and 68
Etretinate and PUVA-dependent psoriatics (Summary), Suppl. 30, 11
Etretinate, low-dose in pustular psoriasis, 577

Facial dermal dysplasia Brauer's syndrome (Case report), Suppl. 30, 43
Fibrosis marker in psoriasis treated with methotrexate (Poster summary), Suppl. 30, 29
Fine needle aspiration cytology in dermatology, 317
Focal dermal hypoplasia (Goltz's syndrome) (Case report), Suppl. 30, 44
Focal dermal hypoplasia (Goltz's syndrome) with haemangiomata (Case report), Suppl. 30, 44

Gamma-glutamyl transpeptidase, a marker for malignant epithelial tumours (Correspondence), 743
Gianotti-Crosti syndrome, 49
Goltz's syndrome (Case report), Suppl. 30, 44
Goltz's syndrome with haemangiomata (Case report), Suppl. 30, 44
Gorlin's syndrome (Case report), Suppl. 30, 95
Granuloma annulare (Case report), Suppl. 30, 82
Granuloma annulare, HLA antigen association, 329
Granuloma annulare, necrobiosis lipoidica and diabetes (Case report), Suppl. 30, 81
Granulomatous cheilitis and positive Kveim test, 619
Gravitational disease, thrombocytosis and raised platelet volume, 521
Gunther's disease (Case report), Suppl. 30, 87

Haemodialysis with bullous eruption (Case report), Suppl. 30, 88
Hailey-Hailey disease (Case report), Suppl. 30, 83
Heat urticaria and prostaglandin $D_2$, 721
*Hendersonula toruloidea* and *Scytalidium hyalinum* antibodies, 435
Hen's egg test (HET)—an alternative toxicity test, Suppl. 31, 133
Hidradenitis suppurativa and androgens, 259
Hidradenitis suppurativa controlled with antiandrogen and oestrogen therapy, 269
Hidradenitis suppurativa, trial with cyproterone acetate, 263
Hidradenomes eruptif (Case report), Suppl. 30, 98
High-pressure injection injury, 379
HTLV III infection with exanthema, 601
HTLV-III virus infection and the skin (Summary), Suppl. 30, 16
Hydrocortisone effects in severe infantile skin disease, 475
Hyperhidrosis of upper limb and surgery, 81
Hyperplasia induced by tape stripping or TPA, Suppl. 31, 128
Hypomelanosis of Ito (Case report), Suppl. 30, 48

Ia antigens induced on rat keratinocytes by γ-interferon, 543
Immunogold labelling of Langerhans cells, 351
Incontinentia pigmenti achromians (Case report), Suppl. 30, 48
Incontinentia pigmenti achromians of Ito (Summary), Suppl. 30, 24
Incontinentia pigmenti and eosinophilic chemotaxis, 61
Incontinentia pigmenti (Case report), Suppl. 30, 47
Indomethacin topically on UVB and UVC radiation, 453
Infantile skin disease and hydrocortisone absorption, 475
Injection, high-pressure injury, 379
Interferon for viral warts (Poster summary), Suppl. 30, 40
Interferon for warts, Suppl. 31, 76
γ-Interferon inducing Ia antigens, 543
Iontophoresis (Correspondence), 516
Irritation of skin measured, Suppl. 31, 45
Ito's disease (Summary), Suppl. 30, 24
Itraconazole for pityriasis versicolor, 217
Itraconazole for *T. rubrum* infections (Poster summary), Suppl. 30, 34

Keratinocytes cultured as a model for environmental modulation, Suppl. 31, 126
Keratosis palmo-plantaris, congenita, pes planus, onychogryphosis, periodontosis, arachnodactyly and a peculiar acro-osteolysis, a new syndrome, 243
Ketanserin effect on Raynaud's phenomenon in scleroderma (Summary), 583
Klippel-Trenaunay-Parkes-Weber syndrome (Case report), Suppl. 30, 49

Langerhans cells, immunogold labelling, 351
Langerhans cells in human warts, 669
Lasers and psoriasis (Correspondence), 386
Lasers, dermal fibroblasts and psoriasis (Correspondence), 744
Laugier-Hunziker syndrome and melanonychia, 707
Leg ulcers and ankle/brachial blood pressure (Summary), Suppl. 30, 26
Leg ulcers and contact sensitivity (Poster summary), Suppl. 30, 41
Leg ulcers treated by skin transplantation (Summary), 584
Leiomyoma cutis (Case report), Suppl. 30, 93
Leukapheresis for T-cell lymphomas, 159
Lichen amyloidosis (Case report), Suppl. 30, 59
Lichen planus and exposure to chemicals, 23
Lichen planus autoimmune background, 199
Lichen planus, oesophageal, 729
Lichen sclerosus et atrophicus in female twins (Correspondence), 743
Linear IgA disease and parasitophobia (Case report), Suppl. 30, 83
Linear juvenile IgA dermatosis and IgA isotypes (Case report), 590

Livedo reticularis (Case report), Suppl. 30, 53
Lupus erythematosus and malabsorption (Case report), Suppl. 30, 56
Lupus erythematosus and morphea in infant with maternal SLE, 85
Lupus erythematosus and papular and nodular mucinosis, 631
Lupus erythematosus and protein losing enteropathy (Case report), Suppl. 30, 58
Lupus erythematosus profundus (Case report), Suppl. 30, 55
Lupus erythematosus treated with oral gold, 211
Lupus miliaris disseminatus faciei lysozyme immunohistochemistry, 187
Lyell's syndrome (Summary), Suppl. 30, 25
Lymphomatoid granulomatosis (Case report), Suppl. 30, 72
Lymphomatoid granulomatosis and natural killer cells (Correspondence), 514
Lymphomatoid papulosis and poikiloderma (Case report), Suppl. 30, 68
Lymphomatoid papulosis and the hypereosinophilic syndrome (Summary), Suppl. 30, 18

Marie-Unna syndrome (Poster summary), Suppl. 30, 32
Mastocytosis, systemic (Case reports), Suppl. 30, 62, 64
Mechlorethamine and PUVA for psoriasis, Suppl. 31, 82
Melanocytic naevus, atypical (Case report), Suppl. 30, 103
Melanocytes, cultured, and plasminogen, 205
Melanoma, acne and psoriasis not associated, 573
Melanonychia and Laugier-Hunziker syndrome, 707
Methotrexate and pulmonary function (Summary), Suppl. 30, 13
Methotrexate damaged liver, ultrasound and radioisotope scans (Summary), Suppl. 30, 13
Methotrexate for benign familial chronic pemphigus (Correspondence), 640
Methotrexate, long-term low-dose and pulmonary function, 657
Methotrexate therapy, haematological consequences (Correspondence), 253
8-Methoxypsoralen dissolution rates compared (Summary), 583
1-Methylquinoxalinium-*p*-toluenesulphonate causing allergic contact dermatitis (Summary), Suppl. 30, 26
Microcomputerization for contact dermatitis (Poster summary), Suppl. 30, 36
Microcystic adnexal carcinoma, 101
Mite antigen percutaneously and atopic dermatitis, 485
Moh's surgery in the N.H.S. (Summary), Suppl. 30, 24
Molluscum contagiosum viral colony sac demonstrated, 557
Monoclonal antibodies assessed in cutaneous infiltrates, 151
Morphea and neonatal lupus erythematosus with maternal SLE, 85
Morphoea, atrophic (Case report), Suppl. 30, 81
Morphoea, localized and auto-immune disorders (Summary), Suppl. 30, 19
Morphoea profunda (Poster summary), Suppl. 30, 37
Mucinosis, papular and nodular and systemic lupus erythematosus, 631
Mucinosis, reticular erythematous (Case report), Suppl. 30, 65
Multicentric reticulohistiocytosis, 623
Mupirocin in atopic dermatitis (Summary), Suppl. 30, 27
Mycosis fungoides and poikiloderma (Summary), Suppl. 30, 17
Myelomonocytic leukaemia and cold sensitivity, 607
Myxoma, atrial metastasizing, 239

Necrobiosis lipoidica, granuloma annulare and diabetes (Case report), Suppl. 30, 81
Needle aspiration in dermatology, 317
Neurofibromatosis (Case report), Suppl. 30, 92
News and notices, 122, 258, 388, 520, 645, 750
Nickel dermatitis and oral challenge, 33
Nicotinamide for polymorphous light eruption, 77
Nifedipine orally in Raynaud's disease (Poster summary), Suppl. 30, 37
Nodular cutaneous chain (Poster summary), Suppl. 30, 32

Obituaries
  Pinkus, Hermann, 507
  Whittle, Claude Howard, 741
Ocular and oral involvement in pemphigoid (Summary), Suppl. 30, 19
Oral and ocular involvement in pemphigoid (Summary), Suppl. 30, 19
Orf, giant, in a normal individual, 497
Ornithine decarboxylase activity in psoriatic skin, 193

Papillomavirus infections and renal transplant recipients, 681
Paracetamol fixed drug eruption, 357
Parasitophobia and linear IgA disease (Case report), Suppl. 30, 83
Pemphigoid and oral and ocular involvement (Summary), Suppl. 30, 19
Pemphigus, benign familial (Hailey-Hailey disease) (Case report), Suppl. 30, 83
Pemphigus, benign familial chronic, treated with methotrexate (Correspondence), 640
Pemphigus foliaceus, Brazilian, thymosin alpha 1 levels, 147
Percutaneous absorption of drugs and repeated administration, Suppl. 31, 49
Percutaneous absorption of topical anti-androgens, Suppl. 31, 36
Percutaneous absorption of topical drugs and their toxicity, Suppl. 31, 71
Percutaneous absorption, vehicle effects, 1
Percutaneous penetration, animal models, 335
Perineural xanthoma, 715
Periungual and subungual arteriovenous tumours, 361
Peutz-Jegher's syndrome without polyposis (Case report), Suppl. 30, 104
Phagocyte potentiated reactive oxidant generation, 285
Phakomatosis pigmentovascularis type IVb (Case report), 589
Photochemotherapy for cutaneous T-cell lymphoma (Summary), Suppl. 30, 17

Photochemotherapy safety, an eight-year follow-up (Summary), Suppl. 30, 14
Photosensitivity due to retinoids, 275
Phototoxic plants in Britain (Poster summary), Suppl. 30, 35
Phototoxicity reactions in skin, Suppl. 31, 86
Pityriasis lichenoides and mycosis fungoides (Case report), Suppl. 30, 69
Pityriasis-like lesions and poikiloderma (Summary), Suppl. 30, 17
Pityriasis versicolor treated with itraconazole, 217
Plasminogen activator in cultured melanocytes, 205
Plasminogen activator inhibitor secreted by cultured epidermal cells, 133
Platelet factors and outgrowth from skin explants, 529
Poikiloderma vasculare atrophicans granulomatosa (Correspondence), 383
Polyarteritis nodosa and angiomatosis, 611
Polymorphous light eruption treated with nicotinamide, 77
Porphyria, congenital erythropoietic (Case report), Suppl. 30, 87
Prostaglandin $D_2$ in localized heat urticaria, 721
Psoriasis, acne and malignant melanoma not associated, 573
Psoriasis and alcohol consumption (Summary), Suppl. 30, 11
Psoriasis and calmodulin levels (Poster summary), Suppl. 30, 30
Psoriasis and cyclosporin A (Correspondence), 249, 747
Psoriasis and lasers (Correspondence), 386, 746
Psoriasis and skin ornithine decarboxylase activity, 193
Psoriasis and Vitamin $D_3$, 421
Psoriasis, palmoplantar pustular and low-dose etretinate, 577
Psoriasis, pustular, precipitated by trazodone, 629
Psoriasis, PUVA-dependent and etretinate (Summary), Suppl. 30, 11
Psoriasis treated with methotrexate, fibrosis marker (Poster summary), Suppl. 30, 29
Psoriasis treated with PUVA and mechlorethamine, Suppl. 31, 82
Psoriasis treated with topical vitamin $D_3$, 431
Pulmonary function and methotrexate (Summary), Suppl. 30, 13
PUVA-dependent psoriatics and etretinate (Summary), Suppl. 30, 11
Pyoderma gangrenosum following subcorneal pustular dermatosis (Correspondence), 117

Raynaud's phenomenon and ketanserin (Summary), 583
Renal transplants and warts, 681
Reticular erythematous mucinosis (Case report), Suppl. 30, 65
Reticulohistiocytosis, multicentric, 623
Retinoids causing photosensitivity, 275

Salbutamol in atopic dermatitis (Poster summary), Suppl. 30, 33
SALT hypothesis evaluated, 389
Sarcoidosis (Case reports), Suppl. 30, 76, 77
Sarcoidosis, cutaneous (Case report), Suppl. 30, 75
Scar tissue, hypertrophic, TIMP and collagenase, 409
Scl 70 antibody, a specific marker of systemic sclerosis, 393
Sclerodactyly, diabetic (Summary), Suppl. 30, 21
*Scytalidium hyalinum* and *Hendersonula toruloidea* infections and antibodies, 435
Seborrhoeic eczema, ?atopic eczema, 341
Sensitization to DNCB, effect of area and dose, 663
Skin blanching test (Correspondence), 637
Skin disease in infants and hydrocortisone absorption, 475
Skin diseases and behaviour variables (Summary), 586
Skin immune system (Summary), 584
Skin irritation measured, Suppl. 31, 45
Skin manifestations of AIDS (Summary), 585
Skin sensitivity in animal models, Suppl. 31, 24
Skin toxicity and cellular metabolism models, Suppl. 31, 108
Skin tumours excised without wound closure, 563
Smooth muscle hamartoma, 711
Solar keratoses, spontaneous remission, 649
Spironolactone orally for female acne, 227
Spreading factors in epidermal cells, 13
*Staphylococcus aureus* infection and erythroderma, 693
Steatocystoma multiplex and pachyonychia congenita in siblings (Case report), Suppl. 30, 99
Stratum corneum reservoir and absorption, 233
Subcorneal pustular dermatosis and pyoderma gangrenosum (Correspondence), 117
Subungual and periungual arteriovenous tumours, 361
Surfactant, amphoteric, on the skin barrier, Suppl. 31, 138
Sweet glands isolated and stimulated, 39
Sweet's syndrome and secondary syphilis, 495
Syphilis mimicking Sweet's syndrome, 495
Systemic sclerosis, Scl 70 antibody specific, 393

Tanning with UV-A sunbeds, 67
T-cell lymphoma and photochemotherapy (Summary), Suppl. 30, 17
T-cell lymphomas treated by leukapheresis, 159
T cells, epidermotropic, do they exist in human skin? 389
T6 antigen and epidermal keratinocytes (Correspondence), 511, 512
T6 antigen and epidermal keratinocytes (Correspondence reply), 513
TCDO and wound healing, Suppl. 31, 142
Thalidomide effect on arachidonic acid metabolism, 677
Thalidomide in neuro-Behçet's syndrome, 367
TIMP and collagenase in hypertrophic scar, 409
Topical drugs, absorption and their toxicity, Suppl. 31, 71
Topical drugs, risks, Suppl. 31, 63
Topical preparation containers and amount used (Correspondence), 743
Toxic epidermal necrolysis and elevated serum amylase (Correspondence), 250
Toxic epidermal necrolysis and elevated serum amylase (Correspondence reply), 250
Trazodone precipitating generalized pustular psoriasis, 629
Trichoepitheliomas, giant solitary in perineum, 91

Trichoepitheliomata with B.C.C. (Case report), Suppl. 30, 95
*Trichophyton rubrum* treated with itraconazole (Poster summary), Suppl. 30, 34
Tricho-rhino-phalangeal syndrome (Case report), Suppl. 30, 45
*Trichosporon beigelii* in Houston (Summary), Suppl. 30, 15
Tropical ulcer and anaerobes (Summary), Suppl. 30, 15

Ulcer, tropical phagedenic and anaerobes (Summary), Suppl. 30, 15
Ultrasound and radio-isotope scans in methotrexate liver damage (Summary), Suppl. 30, 13
Uropygial gland of quail for antiandrogen assays, Suppl. 31, 33
Urticaria, delayed pressure (Summary), Suppl 30, 22
Urticaria, localized heat, and prostaglandin $D_2$, 721
UV-A sunbeds for cosmetic tanning, 67
UVB and UVC radiation and topical indomethacin, 453

Virilism, cutaneous (Poster summary), Suppl. 30, 40
Vitamin $D_3$ in treating psoriasis, 421
Vitamin $D_3$ topically for psoriasis, 431
Vitiligo treated with L-phenylalanine and light (Summary), 587
Vulval conditions, non-neoplastic, 647

Waldenström's hyperglobulinaemic purpura (Case report), Suppl. 30, 91
Wallace's Line in serum sickness and Kawasaki disease (Correspondence), 640
Warts and Langerhans cells, 669
Warts and renal transplants, 681
Warts treated with human lympoblastoid interferon, Suppl. 31, 76
Warts treated with interferon (Poster summary), Suppl. 30, 40
Wound healing and TCDO, Suppl. 31, 142
Wound healing inhibited by antiseptics, Suppl. 31, 41

Xanthelasma (Case report), Suppl. 30, 90
Xanthoma, perineural, 715
Xanthomata and monoclonal gammopathy (Case report), Suppl. 30, 90

# AUTHORS

Abalain, J.H. *see* Daniel, J.Y.
Adachi, K. *see* Katayama, H.
Adam, A.E. *see* Pether, J.V.S.
Adriaans, B., Drasar, B. and Hay, R. The role of anaerobic bacteria in the pathogenesis of tropical (phagedenic) ulcer (Summary) Suppl. 30, 15
Allen, B.R. *see* Maurice, P.D.L.
Allen, M.H. *see* Cerio, R.
Alstadt, S.P. *see* Hebda, P.A.
Amet, Y. *see* Daniel, J.Y.
Anderson, R. and Eftychis, H.A. Potentiation of the generation of reactive oxidants by human phagocytes during exposure to benoaxprofen and ultraviolet radiation *in vitro*, 285
Anhalt, G.J. *see* Roscoe, J.T.
Aoyagi, T. *see* Kato, N.
Archer, C.B. and MacDonald, D.M. Effects of topical and oral salbutamol therapy in atopic dermatitis (Poster summary), Suppl. 30, 33
Asselineau, D., Bernard, B.A., Bailly, C. and Darmon, Y.M. Three-dimensional culture of human keratinocytes on a dermal equivalent as a model system to study environmental modulation of epidermal physiology *in vitro*: effect of air exposure, Suppl. 31, 126

Baber, L. *see* Lever, R.
Bailly, C. *see* Asselineau, D.
Baker, H. Haematological consequences of methotrexate therapy (Correspondence), 253
Baker, H. *see* Barth, J.H.
Baker, H. *see* Phillips, T.J.
Bakkers, E.J.M. *see* Sillevis Smitt, J.H.
Baran, R. and Barrière, H. Longitudinal melanonychia with spreading pigmentation in Laugier-Hunziker syndrome: a report of two cases, 707
Baran, R. *see* Burge, S.M.
Barker, J.N.W.N. and MacDonald, D.M. Epidermal Class II HLA expression in atopic dermatitis (Poster summary), Suppl. 30, 38
Barkley, A.S.J. *see* Maurice, P.D.L.
Barr, R.M. *see* Koro, O.
Barrière, H. *see* Baran, R.
Barrière, H. *see* Dreno, B.
Barth, J.H. and Baker, H. Generalized pustular psoriasis precipitated by trazodone in the treatment of depression, 629
Barth, J.H., Cherry, C.A., Dawber, R.P.R. and Wojnarowska, F. Clinical and laboratory manifestations of cutaneous virilism (Poster summary), Suppl. 30, 40
Beck. M. *see* Shaw, S.
Bellosta, M. *see* Borroni, G.
Belter, S.V., Knop, J., Bruske, K. and Sorg, C. Leukapheresis in the treatment of cutaneous T-cell lymphomas, 159
Benassi, L. *see* Pincelli, C.
Berardesca, E. *see* Borroni, G.
Berberian, B.J. and Burnett, J.W. Congenital smooth muscle hamartoma: a case report, 711
Bergman, R. *see* Friedman-Birnbaum, R.
Bernard, B.A. *see* Asselineau, D.
Bernengo, M.G. *see* Rongioletti, F.
Bernhard, J.D., Rhodes, A.R. and Melski, J W. Wallace's Line in serum sickness and Kawasaki disease (Correspondence), 640
Berthou, F. *see* Daniel, J.Y.
Beutner, E.H. *see* Jarzabek-Chorzelska, M.
Beveridge, G W. *see* Dalziel, K.
Bhogal, B. *see* Logan, R A.
Bickers, D.R., Das, M. and Mukhtar, H. Pharmacological modification of epidermal detoxification systems, Suppl. 31, 9
Birnbaum, J.E. *see* Vitale, G.I.
Bittiner, S.B. and Church, R.E. Cutis marmorata telangiectatica congenita (Case report), Suppl. 30, 51
Bittiner, S.B. and Church, R.E. Focal dermal hypoplasia (Goltz's syndrome) (Case report), Suppl. 30, 44
Bittiner, S.B. and Church, R.E. Lymphomatoid papulosis and poikiloderma (Case report), Suppl. 30, 68
Bittiner, S.B. and Church, R.E. Persistent erythema multiforme (Case report), Suppl. 30, 89
Bittiner, S.B. and Harrington, C.I. Hidradenomes eruptif (Case report), Suppl. 30, 98
Bittiner, S.B. and Harrington, C.I. Primary amyloidosis (Case report), Suppl. 30, 61
Bittiner, S.B. *see* Bleehen, S.S.
Bittiner, S.B. *see* Thomas, S.E.
Bittiner, S.B. *see* Tucker, W.F.G.
Black, M.M. *see* Fenton, D.A
Black, M.M. *see* Logan, R.A.
Black, M.M. *see* Newton, J.A.
Blaszczyk, M. *see* Jarzabek-Chorzelska, M.
Bleehen, S S. Focal dermal hypoplasia (Goltz's syndrome) with extensive capillary haemangioma (Case report), Suppl. 30, 44
Bleehen, S.S. and Slater, D.N. Pityriasis lichenoides developing into mycosis fungoides (Case report), Suppl. 30, 69
Bleehen, S.S. and Strachan, I.M. Neurofibromatosis (Case report), Suppl. 30, 92
Bleehen, S.S. and Wood, M.L Incontinentia pigmenti. Bloch-Sulzberger syndrome (Case report), Suppl. 30, 47
Bleehen, S.S., Bittiner, S.B. and Priestley, B.L. Incontinentia pigmenti achromians (hypomelanosis of Ito) (Case report), Suppl. 30, 48
Bleehen, S.S., Bittiner, S.B. and Priestley, B.L. Incontinentia pigmenti achromians of Ito. A neurocutaneous disorder (Summary), Suppl. 30, 24
Bleehen, S.S. *see* Messenger, A.G.
Bleehen, S.S. *see* Thomas, S.E.
Bleehen, S.S. *see* Tucker, W.F.G.

Bleehen, S.S. *see* Wood, M.L.

Bogman, M.J.J.T. *see* van de Kerkhof, P.C.M.

Bojar, R.A. *see* Leeming, J.P.

Boone, R.M. *see* Ramelsaar, C.G.

Borgers, M. *see* del Palacio Hernanz, A.

Borroni, G., Berardesca, E., Gabba, P., Pericoli, R., Pugliese, P.T., Bellosta, M. and Rabbiosi, G. Effects of an amphoteric surfactant on the skin barrier: an *in vivo* electrophysiological study, Suppl. 31, 138

Bos, J.D. and Kapsenberg, M.L. Skin immune system (SIS) (Summary), 584

Botham, P.A. *see* Oliver, G.J.A.

Bouclier, M., Elbaze, P., Milano, G., Shroot, B. and Ortonne, J-P. Hyperinduction of skin ornithine decarboxylase activity in psoriatic patients, 193

Bouton, M.M. *see* Daniel, J.Y.

Bowcock, S. *see* Meyrick Thomas, R.H.

Boyle, J. Amiodarone-induced cutaneous photosensitivity (Correspondence), 253

Brain, A. *see* Phillips, T.

Brandon, D.R. *see* Willis, C.M.

Breathnach, S.M. Do epidermotropic T cells exist in normal human skin? A re-evaluation of the SALT hypothesis, 389

Bron, A.J. *see* Venning, V.A.

Bronaugh, R.L. and Franz, T.J. Vehicle effects on percutaneous absorption: *in vivo* and *in vitro* comparisons with human skin, 1

Bruske, K. *see* Belter, S.V.

Bucks, D.A. *see* Courtheoux, S.

Bunney, M.H. *see* Rüdlinger, R.

Bureau, B. *see* Dreno, B.

Burge, S.M., Baran, R., Dawber, R.P.R. and Verret, J.L. Periungual and subungual arteriovenous tumours, 361

Burmeister, V. *see* Shelley, W.B.

Burnett, J.W. *see* Berberian, B.J.

Burrows, D. *see* Podmore, P.

Cameron, A.E.P. *see* Malone, P.S.

Campbell, A.C. *see* Shuttleworth, D.

Carol-Murillo, J. *see* Pedragosa, R.

Carter, D.M. *see* Hashimoto, K.

Carter, G.D. *see* Muhlemann, M.F.

Cartwright, P.H. *see* Dalziel, K.

Cauwenbergh, G. *see* del Palacio Hernanz, A.

Cerio, R., Allen, M.H. and MacDonald, D.M. The detection of extracellular antigens in formaldehyde-fixed paraffin-embedded cutaneous tissue (Poster summary), Suppl. 30, 39

Chalmers, R.J.G. *see* Mitchell, D.M.

Chardonnet, Y., Viac, J. and Thivolet, J. Langerhans cells in human warts, 669

Chase, D.G. *see* Heng, M.C.Y.

Cherry, C.A. *see* Barth, J.H.

Cherry, G.W. *see* Kulozik, M.

Chorzelski, T. *see* Jarzabek-Chorzelska, M.

Chow, L. *see* Shaw, S.

Church, R.E. Bazex syndrome (Case report), Suppl. 30, 97

Church, R.E. Plane xanthomata and monoclonal gammopathy (Case report), Suppl. 30, 90

Church, R.E. Reticular erythematous mucinosis (Case report), Suppl. 30, 65

Church, R.E. *see* Bittiner, S.B.

Church, R.E. *see* Thomas, S.E.

Church, R.E. *see* Tucker, W.F.G.

Church, R.E. *see* Wood, M.L.

Church, R.E. Tricho-epitheliomata with basal cell carcinomata (Case report), Suppl. 30, 95

Church, R.E. Xanthelasma (Case report), Suppl. 30, 90

Cilliers, J. *see* Jordaan, H.F.

Clayton, R.J. *see* Walker, M.M.

Clayton, Y.M. *see* Hay, R.J.

Comaish, J.S. *see* Lawrence, C.M.

Comaish, J.S. *see* Puttick, L.

Cook, S.W. *see* Gawkrodger, D.J.

Cooke, E.D. *see* Meyrick Thomas, R.H.

Cormane, R.H. *see* Stolk, L.M.L.

Cormane, R.H., Siddiqui, A.H., Westerhof, W., Schutgens, R.H.B., Hu, R. and Mohan, V.I. Treatment of vitiligo with L-phenylalanine and light (Summary), 587

Cotterill, J.A. *see* Sheehan-Dare, R.A.

Coulomb, B. *see* Saiag, P.

Courtheoux, S., Pechenot, D., Bucks, D.A., Marty, J.P., Maibach, H.I. and Wepierre, J. Effect of repeated skin administration on *in vivo* percutaneous absorption of drugs, Suppl. 31, 49

Cox, N.H., Downey, D.J., Tuyp, E.J., Jones, S.K., Jay, J L., Moseley, H. and MacKie, R.M. Safety of photochemotherapy—an eight-year follow-up study (Summary), Suppl. 30, 14

Cox, N.H., Mitchell, J.N.S. and Morley, W.N. Lichen sclerosus et atrophicus in non-identical female twins (Correspondence), 743

Cream, J.J. *see* Muhlemann, M.F.

Crovato, F. *see* Rongioletti, F.

Cunliffe, W.J. Acne and unemployment (Correspondence), 386

Cunliffe, W.J. *see* Warin, R.P.

Curley, R.K. *see* Macfarlane, A.W.

Dahl, M.G.C. and Malcolm, A.J. Adapting Moh's micrographic surgery to the needs and resources of the National Health Service (Summary), Suppl. 30, 24

Dahl, M.G.C. *see* Lawrence, C.M.

Dalziel, K., Going, G., Cartwright, P.H., Marks, R., Beveridge, G.W. and Rowell, N.R. Treatment of chronic discoid lupus erythematosus with an oral gold compound (auranofin), 211

Daniel, J.Y., Abalain, J.H., Amet, Y., di Stefano, S , Berthou, F., Tea, K., Floch, H.H., Bouton, M.M. and Secchi, J. The quail uropygial gland for assays of systemic antiandrogen side-effects of topical drugs, Suppl. 31, 33

Darmon, Y.M. *see* Asselineau, D.

Das, A.K. *see* Logan, R.A.

Das, M. *see* Bickers, D.R.

Davies-Jones, G.A.B. *see* Thomas, S.E.

Dawber, R.P.R. *see* Barth, J.H.

Dawber, R.P.R. *see* Burge, S.M.

Dawber, R.P.R. *see* Mortimer, P.S.

Dawson, R.A. *see* Tucker, W.F.G.

De Panfilis, G., Manara, G.C. and Ferrari, C. Immunogold labelling of epidermal Langerhans cells on tissue sections of normal human skin, 351

De Wit, F.S. and Hulsmans, R.F.H.J. Hereditary papulo translucent acrokeratoderma as a variant of and in association with palmoplantar punctate keratosis (Case report), 589

De Wolf-Peeters, C. *see* Drijkoningen, M.

Del Palacio Hernanz, A., Frias-Iniesta, J., Gonzalez-Valle, O., Borgers, M., van Cutsem, J. and Cauwenbergh, G. Intraconazole therapy in pityriasis versicolor, 217

Delleman, J.W. *see* Hulsmans, R F.H.J.

Delvoye, P., Mauch, C., Krieg, T. and Lapiere, C.M. Contraction of collagen lattices by fibroblasts from patients and animals with heritable disorders of connective tissue, 139

Desmet, V. *see* Drijkoningen, M.

Di Stefano, S. *see* Daniel, J.Y.

Diaz, L.A. *see* Roscoe, J.T.

Diffey, B.L. Use of UV-A sunbeds for cosmetic tanning, 67

Diffey, B.L. *see* Farr, P.M.

Dodd, H.J. *see* Tatnall, F.M.

Dover, J.S., Kobza Black, A. and Greaves, M.W. A clinical and laboratory study of delayed pressure urticaria (Summary), Suppl. 30, 22

Dover, J.S. *see* Koro, O.

Downey, D. *see* Lever, R.

Downey, D.J. *see* Cox, N.H.

Dowd, P.M., Gaywood, I.C., Kurtz, A.B. and Shipley, M.E Diabetic sclerodactyly (Summary), Suppl. 30, 21

Drasar, B. *see* Adriaans, B.

Dreno, B., Gandon, P., Bureau, B , Milpied, N. and Barrière, H. Skin lesions from hypersensitivity to cold during chronic myelomonocytic leukaemia, 607

Drijkoningen, M., de Wolf-Peeters, C. and Desmet, V. Expression of T6 antigen on the epidermal keratinocytes in various dermatoses (Correspondence), 511

Dubertret, L. *see* Saiag, P.

Dunkel, V.C. Endpoints in short-term tests: significance for carcinogenic potential, Suppl. 31, 117

Dunsmore, I.R. *see* Harrington, C.I.

Du Pasquier, P. *see* Taieb, A.

Dupuis, D., Rougier, A., Roguet, R. and Lotte, C. The measurement of the stratum corneum reservoir a simple method to predict the influence of vehicles on *in vivo* percutaneous absorption, 233

Eady, R.A.J. *see* Heagerty, A.H.M.

Eady, R.A.J. *see* Mayou, S.C.

Eaglstein, W.H. *see* Hebda, P.A.

Ebling, F.J.G. Hidradenitis suppurativa: an androgen-dependent disorder, 259

Ebling, F.J.G. *see* Sawers, R.S.

Eedy, D.J. *see* Podmore, P.

Eftychis, H.A. *see* Anderson, R.

Ehrlich, H.P. *see* Hembrey, R.M.

Ekerdt, T., Opitz, D., Hubner, H., Patzold, C. and Topert, M. Percutaneous absorption of topically applied antiandrogens: an assessment with three independent methods, Suppl. 31, 36

Elbaze, P. *see* Bouclier, M.

Elpern, D.J. *see* Walcyk, P.J.

Elwood, J.M., Gallagher, R.P. and Stapleton, P.J. No association between malignant melanoma and acne or psoriasis: results from the Western Canada Melanoma Study, 573

English, J.S.C., Foulds, I., White, I.R. and Rycroft, R.J.G. Allergic contact dermatitis from an epoxy compound in a cutting oil (Poster summary), Suppl. 30, 33

English, J.S.C , White, I.R. and Rycroft, R.J.G. Allergic contact dermatitis from 1-methylquinoxalinium-*p*-toluenesulphonate in a lithographic printing conditioner (Summary), Suppl. 30, 26

Evans, C.D. and Wightman, J. Influence of containers on the quantities of topical preparations used (Correspondence), 743

Everard, M. *see* Young, E.

Faber, W.R. Sensory loss following cryosurgery (Summary), 585

Fairris, G.M., White, J.E , Leppard, B.J. and Goodwin, P.G. Methotrexate for intractable benign familial chronic pemphigus (Correspondence), 640

Farr, P.M. and Diffey, B.L. A quantitative study of the effect of topical indomethacin on cutaneous erythema induced by UVB and UVC radiation, 453

Fell, G.S. *see* Gawkrodger, D.J.

Fenton, D.A., Pope, F.M., Temple, A., Galloway, W.A., Gasson, G., Black, M.M. and Wilkinson, J.D. Ehlers Danlos syndrome type VIII (Poster summary), Suppl. 30, 42

Ferguson, J. and Johnson, B.E. Amiodarone-induced cutaneous photosensitivity (Correspondence reply), 254

Ferguson, J. and Johnson, B.E. Photosensitivity due to retinoids: clinical and laboratory studies, 275

Ferguson, J., Walker, E.M. and Johnson, B.E. Further clinical and investigative studies of chlorpromazine phototoxicity (Poster summary), Suppl. 30, 35

Ferrari, C. *see* de Panfilis, G.

Fleury, P. *see* Westerhof, W.

Floch, H.H. *see* Daniel, J.Y.

Foley, P. *see* Marks, R.

Foulds, I. *see* English, J.S.C

Foulds, I.S. *see* Shehade, S.A.

Francis, D.M. *see* Koro, O.

Franz, T.J. *see* Bronaugh, R.L.

Frederiksen, T. *see* Melin, L.

Frias-Iniesta, J. *see* del Palacio Hernanz, A.

Friedman, K., Pollack, S.V., Manning, T and Pinnell, S.R. Degradation of porcine dermal connective tissue by collagenase and hyaluronidase, 403

Friedmann, P.S. *see* White, S.I.

Friedman-Birnbaum, R., Gideoni, O., Bergman, R. and Pollack, S. Study of HLA antigen association in localized and generalized granuloma annulare, 329

Frith, P.A. *see* Venning, V.A.

Frumkin, A. Arterial lesions of the lower lip (Correspondence), 254

Fukuo, K. *see* Morimoto, S.
Fürstenberger, G. *see* Marks, F.

Gabba, P. *see* Borroni, G.
Gales, M.A. *see* Mortimer, P.S.
Gallagher, R.P. *see* Elwood, J.M.
Galloway, W.A. *see* Fenton, D.A.
Gandon, P. *see* Dreno, B.
Gasson, G. *see* Fenton, D.A.
Gawkrodger, D.J., Cook, S.W., Fell, G.S. and Hunter, J.A.A. Nickel dermatitis: the reaction to oral nickel challenge, 33
Gaywood, I.C. *see* Dowd, P.M.
Giacomoni, P.U. Onc-gene expression in hyperplasia induced by tape stripping or by topical application of TPA, Suppl. 31, 128
Gibson, J.R., Harvey, S.G., Kemmett, D. and Salisbury, J. Treatment of common and plantar viral warts with human lymphoblastoid interferon α (Poster summary), Suppl. 30, 40
Gibson, J.R., Harvey, S.G., Kemmett, D., Salisbury, J. and Marks, P. Treatment of common and plantar viral warts with human lymphoblastoid interferon α—pilot studies with intralesional, intramuscular and dermojet injections, Suppl. 31, 76
Gideoni, O. *see* Friedman-Birnbaum, R.
Gilchrest, B.A. Defined culture systems for pharmacological and toxicological studies, Suppl. 31, 17
Giles, P.J. *see* Vitale, G.I.
Girolomoni, G. *see* Pincelli, C
Gobel, C. *see* Shaw, S.
Goodfield, M.J.D. A relative thrombocytosis and elevated mean platelet volume are features of gravitational disease, 521
Goodman, G. *see* Marks, R.
Going, G. *see* Dalziel, K.
Goldstein, A.L. *see* Roscoe, J.T.
Gollhausen, G.S. *see* Kligman, A.
Gondo, A., Saeki, N. and Tokuda, Y. Challenge reactions in atopic dermatitis after percutaneous entry of mite antigen, 485
Gonzalez-Valle, O. *see* del Palacio Hernanz, A.
Goodwin, P G. *see* Fairris, G.M.
Graham, R.M. *see* Macfarlane, A.W.
Graham-Brown, R.A.C *see* Lister, D.M.
Graham-Brown, R.A.C. *see* Shuttleworth, D.
Greaves, M.W. *see* Dover, J.S.
Greaves, M.W. *see* Koro, O.
Greaves, M.W. *see* Mayou, S.C.
Greaves, M.W. *see* Warin, R.P.
Green, C.A., Walker, D.J. and Malcolm, A.J. A case of multicentric reticulohistiocytosis: uncommon clinical signs and a report of T-cell marker characteristics, 623
Grekin, R.C. *see* Swanson, N.A.
Griffiths, C.E.M. *see* Walker, M.M.
Griffiths, W.A.D. *see* Logan, R.A.
Grimes, S.M. *see* Meyrick Thomas, R.H.
Grosshans, E. *see* Rongioletti, F.
Guerrier, C.J.W. *see* Pether, J.V.S.
Guillaume, J.C. *see* Roujeau, J.C.
Guillet, G. *see* Taieb, A.
Gummer, C.L. and Maibach, H.I. Animal models for percutaneous penetration: assessing contamination of the experimental system, 335

Haboubi, N.Y. *see* Mitchell, D.B.
Hackett, M. *see* Phillips, T.
Hadley, K. *see* Lever, R.
Hage, B.H. *see* Marks, R.
Halprin, K.M. *see* Katayama, H.
Halprin, K.M. *see* Kato, N.
Hannigan, C. *see* Lever, R.
Hardwick, N. and Saxe, N. Patterns of dermatology referrals in a general hospital, 167
Harrington, C.I. Epidermal bullosa simplex. Mental and physical handicap (Case report), Suppl. 30, 84
Harrington, C.I. Focal facial dermal dysplasia (Brauer's syndrome) (Case report), Suppl. 30, 43
Harrington, C.I. Gunther's disease (congenital erythropoietic porphyria) (Case report), Suppl. 30, 87
Harrington, C.I. Peutz-Jegher's syndrome without polyposis (Case report), Suppl. 30, 104
Harrington, C.I. *see* Bittiner, S.B.
Harrington, C.I. *see* Maloney, C.
Harrington, C.I. *see* Tucker, W.F.G.
Harrington, C.I. *see* Wood, M.L.
Harrington, C.I. and Dunsmore, I.R. An investigation into the incidence of auto-immune disorders in patients with localized morphoea (Summary), Suppl. 30, 19
Harrington, C.I. and Messenger, A.G. Birt-Hogg-Dubé syndrome and lichen sclerosis et atrophicus (Case report), Suppl. 30, 97
Harris, W. *see* Walker, M.M.
Harrison, P.V. Lasers and psoriasis (Correspondence), 386
Harvey, S.G. *see* Gibson, J.R.
Hashimoto, K., Horikoshi, T., Nishioka, K., Yoshikawa, K. and Carter, D.M. Plasminogen activator secreted by cultured human melanocytes, 205
Hashimoto, K., Nishioka, K., Katayama, I. and Yoshikawa, K. Secretion of plasminogen activator inhibitor by cultured human epidermal cells, 133
Hay, R.J., Clayton, Y.M and Midgley, G. The use of itraconazole in chronic *Trichophyton rubrum* infections (Poster summary), Suppl. 30, 34
Hay, R. *see* Adriaans, B.
Hay, R.J. *sse* Moore, M.K.
Haynes, H.A. *see* Horan, R.F.
Heagerty, A.H.M., Kennedy, A.R., Leigh, I.M., Purkis, P. and Eady, R.A.J. Identification of an epidermal basement membrane defect in recessive forms of dystrophic epidermolysis bullosa by LH7:2 monoclonal antibody: use in diagnosis, 125
Heagerty, A.H.M., Tidman, M.J., Leigh, I.M., Jassima. and Eady, R.A.J. Dystrophic epidermolysis bullosa: monoclonal antibodies reveal heterogeneity within clinical groups (Summary), Suppl. 30, 20
Hebda, P.A., Alstadt, S.P., Hileman, W T. and Eaglstein, W.H. Support and stimulation of epidermal cell outgrowth from porcine skin explants by platelet factors, 529
Hembry, R.M. and Ehrlich, H.P Immunolocalization

of collagenase and tissue inhibitor of metalloproteinases (TIMP) in hypertrophic scar tissue, 409

Heng, M.C.Y., Kloss, S.G. and Chase, D.G. Erythroderma associated with mixed lymphocyte-endothelial cell interaction and *Staphylococcus aureus* infection, 693

Hernandez, J.V. *see* Pendragosa, R

Hileman, W.T. *see* Hebda, P.A.

Holdsworth, C.D. *see* Thomas, S.E.

Holland, K.T. *see* Leeming, J.P.

Horan, R.F., Tahan, S.R., Margolis, R.J. and Haynes, H.A. Atypical angiomatosis in polyarteritis nodosa, 611

Horikoshi, T. *see* Hashimoto, K.

Hu, R. *see* Cormane, R.H.

Hubner, H. *see* Ekerdt, R.

Hunter, J.A.A. *see* Gawkrodger, D.J.

Hulsebosch, H.J. Skin manifestations of the acquired immune deficiency syndrome (AIDS) (Summary), 585

Hulsmans, R.F.H.J. *see* de Wit, F.S.

Hulsmans, R.F.H.J. *see* Kuiters, G.R R.

Hulsmans, R.F.H.J. *see* van der Linden, M.M.D.

Hulsmans, R.F.H.J., van Schooneveld, M.J., Klop, K., Sillevis Smitt, J.H., Delleman, J.W. and Westerhof, W. Ectrodactyly, ectodermal dysplasia and cleft lip-palate syndrome (EEC syndrome) (Case report), 587

Hunter, J.A.A. *see* Rudlinger, R.

Hussain, S.T. Gamma-glutamyl transpeptidase as a marker for distinguishing malignant epithelial tumours (Correspondence), 743

Imanaka, S. *see* Morimoto, S.

Ince, P. *see* Puttick, L.

Isoda, M. *see* Mihara, K.

Itami, S. *see* Katayama, H.

Iwatsuki, K. *see* Tagami, H.

Jablonska, S. *see* Jarzabek-Chorzelska, M.

Jageneau, A.H.M. *see* van der Linden, M.M.D.

Jansen, F.C. Treatment of leg ulcers by skin transplantation (Summary), 584

Jarzabek-Chorzelska, M., Blaszczyk, M., Jablonska, S., Chorzelski, T., Kumar, V. and Beutner, E.H Scl 70 antibody—a specific marker of systemic sclerosis, 393

Jassim, A. *see* Heagerty, A.H.M.

Jay, J L. *see* Cox, N.H.

Johansson, C. *see* Scheynius, A.

Johnson, B.E. *see* Ferguson, J.

Johnson, R.J. *see* Mitchell, D.M.

Jones, D.H. *see* Phillips, T.J.

Jones, S.K. *see* Cox, N.H.

Jones, S.M. *see* Pether, J.V.S.

Jordaan, H.F. and Cilliers, J. Secondary syphilis mimicking Sweet's syndrome, 495

Kalter, D.C. The epidemiology of *Trichosporon beigelii* infection in Houston, Texas (Summary), Suppl. 30, 15

Kamido, H. *see* Okada, N

Kapsenberg, M.L. *see* Bos, J.D.

Katayama, H., Kino, J., Itami, S., Tsutsui, M., Koizumi, H., Halprin, K.M. and Adachi, K. Monoclonal antibodies against epidermal cell surface and dermal-epidermal junction: antigens which may be epidermal cell spreading factors, 13

Katayama, I. *see* Hashimoto, K.

Katayama, S. *see* Okada, N.

Kato, T., Rokugo, M., Terui, T. and Tagami, H. Successful treatment of psoriasis with topical application of active vitamin $D_3$ analogue, 1α, 24-dihydroxycholecalciferol, 431

Kato, N., Suya, H., Aoyagi, T., Taylor, J.R. and Halprin, K.M. Ionophore A23187 stimulates arachidonic acid release and cyclic GMP accumulation in pig epidermis, 297

Kelly, R.W. *see* Koro, O.

Kemmett, D. *see* Gibson, J.R.

Kennedy, A.R. *see* Heagerty, A.H.M.

Kesseler, M.E. Dowling-Degos disease, Suppl. 30, 103

Kesseler, M.E. Steatocystoma multiplex suppurativa and pachyonychia congenita in two siblings (Case report), Suppl. 30, 99

Kesseler, M.E. *see* Wood, M.L.

Kesseler, M.E. and Slater, D. Atypical dermal melanocytic naevus (Case report), Suppl. 30, 103

Kesseler, M.E. and Slater, D. Non-iatrogenic chronic arcenicism (Case report), Suppl. 30, 102

Kimber, I. *see* Oliver, G.J.A.

Kingsbury, W.N. *see* Pether, J.V.S.

Kingston, T. and Lowe, N.J. Factors influencing anthralin irritancy, Suppl. 31, 80

Kino, J. *see* Katayama, H.

Kirby, J.D.T. *see* Meyrick Thomas, R.H.

Kitano, Y. *see* Morimoto, S.

Kitano, Y. *see* Okada, N.

Kligman, A. and Gollhausen, R. The 'angry back': a new concept or old confusion? Suppl. 31, 93

Klop, K. *see* Hulsmans, R.F.H.J.

Kloss, S.G. *see* Heng, M.C.Y.

Kluin-Nelemans, H.C. *see* Ramelsaar, C G.

Knop, J. *see* Belter, S.V.

Kobza Black, A. *see* Dover, J.S.

Kobza Black, A. *see* Koro, O.

Kobza Black, A. *see* Mayou, S.C.

Koh, E. *see* Morimoto, S.

Koizumi, H. *see* Katayama, H.

Koro, O., Dover, J.S., Francis, D.M , Kobza Black, A., Kelly, R.W., Barr, R.M. and Greaves, M.W. Release of prostaglandin $D_2$ and histamine in a case of localized heat urticaria, and effect of treatments, 721

Kovaks, I.B. *see* Meyrick Thomas, R.H

Koya, M. *see* Ohtaki, N.

Kozuka, T. *see* Morimoto, S.

Krieg, T. *see* Delvoye, P.

Kuhne, F.W. *see* Stahl, K.W.

Kuiters, G.R.R., Hulsmans, R.F.H.J. and Lichtveld, P.L.M. Erosive adenomatosis of the nipple (Case report), 594

Kulozik, M., Cherry, G.W. and Ryan, T.J. The importance of measuring the ankle/brachial systolic pressure ratio in the management of leg ulcers (Summary), Suppl. 30, 26

Kulozik, M., Powell, S , Ryan, T.J. and Cherry, G.

Contact sensitivity in community-based leg ulcer patients (Poster summary), Suppl. 30, 41
Kumahara, Y. *see* Morimoto, S.
Kumar, V. *see* Jarzabek-Chorzelska, M.
Kurtz, A.B. *see* Dowd, P.M.

Labib, R.S. *see* Roscoe, J.T.
Lammers, A.M., van de Kerkhof, P.C.M., Schalwijk, J. and Mier, P.D. Elastase, a marker for neutrophils in skin infiltrates, 181
Lapiere, C.M. *see* Delvoye, P.
Lavker, R.M., Schechter, N.M. and Lazarus, G.S. Effects of topical corticosteroids on human dermis, Suppl. 31, 101
Lawrence, C.M., Comaish, J.S. and Dahl, M.G.C. Excision of skin tumours without wound closure, 563
Lawrence, C.M. *see* Paramsothy, Y.
Lazarus, G.S. *see* Lavker, R.M.
Leeming, J.P., Holland, K.T. and Bojar, R.A. The *in vitro* antimicrobial effect of azelaic acid, 551
Leigh, I M. *see* Heagerty, A.H.M
Leigh, I.M. *see* Phillips, T.
Leisti, S. *see* Turpeinen, M.
Leonard, J.N. *see* Walker, M.M.
Leppard, B.J. *see* Fairris, G.M.
Leppard, B.J. *see* Marshall, R.J.
Lever, R., Downey, D., Hadley, K., Baber, L., Hannigan, C. and MacKie, R.M. A double-blind study of the effect of topical mupirocin in chronic atopic dermatitis (Summary), Suppl. 30, 27
Lichtveld, P.L.M. *see* Kuiters, G.R.R.
Lidén, C. Lichen planus in relation to occupational and non-occupational exposure to chemicals, 23
Lindhardt, B.Ø. *see* Wantzin, G.R.L.
Lister, D.M. and Graham-Brown, R.A.C Pre-reticulotic eruption with alopecia (Case report), Suppl. 30, 71
Lister, D.M., Shuttleworth, D., Graham-Brown, R.A.C. and Tanner, S. Alagille's syndrome (arteriohepatic dysplasia) (Case report), Suppl. 30, 52
Logan, R.A. and Griffiths, W A.D. Experience with etretinate for 'PUVA-dependent' psoriatics (Summary), Suppl. 30, 11
Logan, R.A., Bhogal, B., Das, A.K., McKee, P.H. and Black, M.M. Localization of bullous pemphigoid antibody—an indirect immunofluorescence study of 212 cases using a split-skin technique (Poster summary), Suppl. 30, 29
Logan, R.A., Spittle, M.F. and Smith, N.P. Photochemotherapy for cutaneous T-cell lymphoma—the St John's experience (Summary), Suppl. 30, 17
Lombard, A. Evaluation and relevance of skin sensitivity in animal models, Suppl. 31, 24
Lotte, C. *see* Dupuis, D.
Lovell, C.R. and Machin, G. Phototoxic plants in Britain (Poster summary), Suppl. 30, 35
Low, J. Iontophoresis (Correspondence), 516
Lowe, N.J. Cutaneous phototoxicity reactions, Suppl 31, 86
Lowe, N.J. *see* Kingston, T.
Lupke, N.P. The hen's egg test (HET)—an alternative toxicity test, Suppl. 31, 133
MacCaulay, J.C. Occupational high-pressure injection injury, 379
MacDonald, D.M. *see* Archer, C.B
MacDonald, D.M. *see* Barker, J.N.W.N.
MacDonald, D.M. *see* Cerio, R.
MacDonald, D.M. *see* Tidman, M.J.
MacFarlane, A.W., Curley, R.K. and Graham, R.M. Recurrence rates of basal cell carcinoma according to site, method of removal, histological type and adequacy of excision (Summary), Suppl. 30, 23
McGibbon, D.H. *see* Newton, J.A.
McGrath, H. and Slater, D. Waldenstrom's hyperglobulinaemic purpura associated with Sjogren's syndrome (Case report), Suppl. 30, 91
MacKay, A. *see* Meyrick Thomas, R H.
McKee, P.H. *see* Logan, R.A
McKenzie, A.W. First blast of the trumpet against the monstrous regiment of scientists (Correspondence), 637
MacKie, R.M. *see* Cox, N.H.
MacKie, R.M. *see* Lever, R.
MacKie, R.M. *see* Turbitt, M.L.
MacNeil, S *see* Tucker, W.F.G.
Machin, G. *see* Lovell, C.R.
Mahood, J. *see* Thomas, S.E.
Maibach, H.I. *see* Courtheoux, S.
Maibach, H.I. *see* Gummer, C.L.
Malcolm, A.J. *see* Dahl, M.G.C.
Malcolm, A.J. *see* Green, C.A.
Maleville, J. *see* Taieb, A.
Malone, P.S., Cameron, A.E.P. and Rennie, J.A. The surgical treatment of upper limb hyperhidrosis, 81
Maloney, C., Wood, M.L , Spencer, R.C. and Harrington, C.I. The cutaneous nodular chain. an unusual pattern of infection (Poster summary), Suppl. 30, 32
Manara, G.C. *see* de Panfilis, G.
Manning, T. *see* Friedman, K.
Margolis, R.J. *see* Horan, R.F.
Marks, F. and Furstenberger, G. Experimental evidence that skin carcinogenesis is a multistep phenomenon, Suppl. 31, 1
Marks, J.M. Cyclosporin A treatment of severe psoriasis (Correspondence), 745
Marks, J.M. *see* White, S.I.
Marks, P. *see* Gibson, J.R.
Marks, R., Foley, P., Goodman, G., Hage, B.H. and Selwood, T.S. Spontaneous remission of solar keratoses: the case for conservative management, 649
Marks, R. *see* Dalziel, K.
Marks, V.J. and Miller, O.F. Atrophia maculosa varioliformis cutis, 105
Marsden, A. *see* Newton, J.A.
Marshall, R.J. and Leppard, B.J. Arterial lesions of the lower lip (Correspondence reply), 255
Marty, J P. *see* Courtheoux, S.
Matsuo, M. *see* Ohtaki, N.
Matsuzawa, Y. *see* Okada, N.
Mauch, C. *see* Delvoye, P.
Mauduit, G., Salle, G., Schott, A.M. and Thivolet, J Treatment of patients with psoriasis by PUVA therapy and mechlorethamine, Suppl. 31, 82

Maurice, P.D.L. and Allen, B.R. Arachidonic acid metabolism by polymorphonuclear leukocytes in psoriasis (Correspondence reply), 518

Maurice, P.D.L., Barkley, A.S.J. and Allen, B.R. The effect of thalidomide on arachidonic acid metabolism in human polymorphonuclear leukocytes and platelets, 677

Mayou, S.C., Kobza Black, A., Eady, R.A.J. and Greaves, M.W. Cholinergic dermographism, 371

Melin, L., Frederiksen, T., Noren, P. and Swebilius, B.G. Behavioural treatment of scratching in patients with atopic dermatitis, 467

Melski, J.W. *see* Bernhard, J.D.

Messenger, A.G. and Bleehen, S.S. Sarcoidosis (Case report), Suppl. 30, 77

Messenger, A.G. and Church, R.E. Subacute cutaneous lupus erythematosus and malabsorption (Case report), Suppl. 30, 56

Messenger, A.G. for Church, R.E. Darier's disease (Case report), Suppl. 30, 67

Messenger, A.G. and Harrington, C.I. Candida-endocrinopathy syndrome. Squamous cell carcinoma of the tongue (Case report), Suppl. 30, 100

Messenger, A.G. and Walker, A.E. Marie-Unna syndrome (Poster summary), Suppl. 30, 32

Messenger, A.G. *see* Church, R.E.

Meyrick Thomas, R.H. and Munro, D.D. Fixed drug eruption due to paracetamol, 357

Meyrick Thomas, R.H., Rademaker, M., Grimes, S.M., Kovaks, I.B., MacKay, A., Bowcock, S., Cooke, E.D. and Kirby, J.D.T. Oral nifedipine treatment for Raynaud's phenomenon secondary to systemic sclerosis—a double-blind, placebo-controlled study (Poster summary), Suppl. 30, 37

Midgley, G. *see* Hay, R.J.

Mier, P.D. *see* Lammers, A.M.

Mihara, K. and Isoda, M. Immunohistochemical study of lysozyme in lupus miliaris disseminatus faciei, 187

Mihara, M. *see* Nakayama, H.

Milano, G. *see* Bouclier, M.

Millard, P.R. *see* Venning, V.A.

Miller, O.F. *see* Marks, V.J.

Milpied, N. *see* Dreno, B.

Mitchell, D.M., Smith, A., Rowan, B.P., Warnes, T.W., Haboubi, N.Y. and Chalmers, R.J.G. The aminoterminal peptide of type III procollagen as a serological marker of fibrosis in psoriatic patients treated with methotrexate (Poster summary), Suppl. 30, 29

Mitchell, D.M., Johnson, R.J., Testa, H.J., Haboubi, N.Y. and Chalmers, R.J.G. Ultrasound and radioisotope scans are poor indicators of liver damage in psoriatic patients treated with methotrexate (Summary), Suppl. 30, 13

Mitchell, J.N.S. *see* Cox, N.H.

Miyamoto, C. *see* Ohtaki, N.

Mohan, V.I. *see* Cormane, R.H.

Mol, M.A.E., van Genderen, J. and Wolthuis, O.L. An evaluation of two approaches for dermatotoxicity testing, Suppl. 31, 136

Monk, B.E. Cyclosporin A and psoriasis (Correspondence), 249

Monk, B.E. and Neill, S.M. The role of alcohol consumption in the natural history of psoriasis (Summary), Suppl. 30, 11

Moore, M.K. and Hay, R.J. Circulating antibodies and antigenic cross-reactivity in *Hendersonula toruloidea* and *Scytalidium hyalinum* infections, 435

Moore, R.A. *see* Mortimer, P.S.

Morimoto, S., Yoshikawa, K., Kozuka, T., Kitano, Y., Imanaka, S., Fukuo, K., Koh, E. and Kumahara, Y. An open study of vitamin $D_3$ treatment in psoriasis vulgaris, 421

Morley, W.N. *see* Cox, N.H.

Mortimer, P.S., Dawber, R.P.R., Gales, M.A. and Moore, R.A. A double-blind controlled cross-over trial of cyproterone acetate in females with hidradenitis suppurativa, 263

Moseley, H. *see* Cox, N.H.

Moss, C. *see* White, S.I.

Muhlemann, M.F., Carter, G.D., Cream, J.J. and Wise, P. Oral spironolactone: an effective treatment for acne vulgaris in women, 227

Mukhtar, H. *see* Bickers, D.R.

Munro, D.D. *see* Meyrick Thomas, R.H.

Nakamura, T. *see* Okada, N

Nakayama, H., Mihara, M. and Shimao, S. Perineural xanthoma, 715

Navsaria, H. *see* Phillips, T.

Naylor, P.H. *see* Roscoe, J.T.

Neill, S.M. *see* Monk, B.E.

Neumann, R., Rappold, E. and Pohl-Markl, H. Treatment of polymorphous light eruption with nicotinamide: a pilot study, 77

Newton, J.A. and Black, M.M. Degos' disease (Correspondence), 249

Newton, J.A., Salisbury, J., Marsden, A. and McGibbon, D.H. Acropustulosis of infancy, 735

Niedner, R. and Schopf, E. Inhibition of wound healing by antiseptics, Suppl. 31, 41

Nishioka, K. *see* Hashimoto, K.

Noren, P. *see* Melin, L.

Ohara, K. *see* Okada, N.

Ohtaki, N., Miyamoto, C., Orita, M., Koya, M. and Matsuo, M. Concurrent multiple morphea and neonatal lupus erythematosus in an infant boy born to a mother with SLE, 85

Okada, N., Yamamura, T., Kitano, Y., Nakamura, T., Kamido, H., Matsuzawa, Y., Katayama, S and Ohara, K. Metastasizing atrial myxoma: a case with multiple subcutaneous tumours, 239

Oliver, G.J.A., Botham, P.A. and Kimber, I. Models for contact sensitization—novel approaches and future developments, Suppl. 31, 53

Oliver, G.J.A. *see* Pemberton, M.A.

Opitz, D. *see* Ekerdt, R.

Orita, M. *see* Ohtaki, N.

Ortonne, J-P. *see* Bouclier, M.

Paramsothy, Y., Lawrence, C.M. and Shuster, S. Anthralin inflammation: lack of effect of topical thiocarbamide drugs (Correspondence), 251

Patel, H.P. *see* Roscoe, J.T.

Patzold, C. *see* Ekerdt, R.

Pechenot, D. *see* Courtheoux, S.

Pedragosa, R., Serrano, S., Carol-Murillo, J., Hernandez, J.V. and Vidal, J. Blisters over burn scars in a child, 501

Pemberton, M.A. and Oliver, G.J.A. The measurement of skin irritation *in vitro* using *ex vivo* rabbit skin, Suppl. 31, 45

Penso, D. *see* Roujeau, J.C.

Pepper, M. *see* Young, E.

Pericoli, R. *see* Borroni, G

Pether, J.V.S., Guerrier, C.J.W., Jones, S.M., Adam, A.E. and Kingsbury, W N. Giant orf in a normal individual, 497

Phillips, T., Leigh, I.M., Hackett, M., Brain, A., Purkis, P. and Navsaria, H. Allografts of cultured keratinocytes in clinical practice (Summary), Suppl. 30, 21

Phillips, T.J., Wallis, P. and Baker, H. Pulmonary function in patients on long-term, low-dose methotrexate (Summary), Suppl. 30, 13

Phillips, T.J., Wallis, P.J., Jones, D.H. and Baker, H. Pulmonary function in patients on long-term, low-dose methotrexate, 657

Pincelli, C., Girolomoni, G. and Benassi, L. Expression of T6 antigen on the epidermal keratinocytes in various dermatoses (Correspondence), 512

Pinnell, S.R. *see* Friedman, K.

Plantin, P. *see* Taieb, A.

Plasma, A. *see* Stolk, L.M.L.

Podmore, P., Burrows, D., Eedy, D.J. and Stanford, C.F. Seborrhoeic eczema—a disease entity or a clinical variant of atopic eczema? 341

Pohl-Markl, H. *see* Neumann, R.

Pollack, S. *see* Friedman-Birnbaum, R.

Pollack, S.V. *see* Friedman, K.

Pope, F.M. *see* Fenton, D.A.

Powell, S. *see* Kulozik, M.

Powles, A.V. *see* Walker, M.M

Priestley, B.L. *see* Bleehen, S.S.

Pugliese, R. *see* Borroni, G.

Puliyel, J.M. and Sridharan Iyer, K.S. A syndrome of keratosis palmo-plantaris congenita, pes planus, onychogryphosis, periodontosis, arachnodactyly and a peculiar acro-osteolysis, 243

Purkis, P. *see* Heagerty, A.H.M.

Purkis, P. *see* Phillips, T.

Puttick, L., Ince, P. and Comaish, J.S. Three cases of eccrine porocarcinoma, 111

Puttick, L. *see* White, S.I.

Quatrale, R.P. *see* Vitale, G.I.

Rabbiosi, G. *see* Borroni, G.

Rademaker, M. *see* Meyrick Thomas, R.H.

Ramselaar, C.G., Boone, R.M. and Kluin-Nelemans, H.C. Thalidomide in the treatment of neuro-Behçet's syndrome, 367

Ramsey, J *see* Scott, R.C.

Randall, V.A. *see* Sawers, R.S.

Rappold, E. *see* Neumann, R.

Rebora, A. *see* Rongioletti, F.

Reichert, U.W.E. Skin toxicity and cellular metabolism: *in vitro* models, Suppl. 31, 108

Reilly, G. *see* Slater, D.N.

Rennie, J.A. *see* Malone, P.S.

Revuz, J. *see* Roujeau, J.C.

Rhodes, A.R. *see* Bernhard, J.D.

Rhodes, C. *see* Scott, R.C.

Ridley, C.M. Nomenclature of non-neoplastic vulval conditions, 647

Rivers, J.K., Samman, P.D., Spittle, M F. and Smith, N.P. Pityriasis lichenoides-like lesions associated with poikiloderma: a precursor of mycosis fungoides (Summary), Suppl. 30, 17

Roguet, R. *see* Dupuis, D.

Rokugo, M. *see* Kato, T.

Rongioletti, F. and Rebora, A. Papular and nodular mucinosis associated with systemic lupus erythematosus, 631

Rongioletti, F., Bernengo, M.G., Crovato, F. and Rebora, A. Natural killer cells and lymphomatoid granulomatosis (Correspondence), 514

Rongioletti, F., Grosshans, E. and Rebora, A. Microcystic adnexal carcinoma, 101

Roscoe, J.T., Naylor, P.H., Diaz, L.A., Labib, R.S., Patel, H.P., Goldstein, A.L., Sampaio, S.A.P. and Anhalt, G.J. Elevated thymosin alpha 1 levels in Brazilian pemphigus foliaceus, 147

Rougier, A. *see* Dupuis, D.

Roujeau, J.C., Rowland Payne, C.M.E., Guillaume, J.C., Penso, D., Saiag, P., Revuz, J. and Touraine, R. Lyell's syndrome—Créteil's experience in 87 cases (Summary), Suppl. 30, 25

Rowan, B.P. *see* Mitchell, D.M.

Rowell, N.R. *see* Dalziel, K.

Rowland Payne, C.M.E. *see* Roujeau, J.C.

Rowland Payne, C.M.E. *see* Saiag, P.

Rudlinger, R., Smith, I.W., Bunney, M.H. and Hunter, J.A.A. Human papillomavirus infections in a group of renal transplant recipients, 681

Russell Jones, R. *see* Whittaker, S.

Ruzicka, T. Arachidonic acid metabolism by polymorphonuclear leukocytes in psoriasis (Correspondence), 517

Ryan, T.J. *see* Kulozik, M.

Ryan, T.J. *see* Venning, V.A.

Rycroft, R.J.G. *see* English, J.S.C.

Saeki, N. *see* Gondo, A.

Saiag, P., Coulomb, B., Rowland Payne, C.M.E., Dubertret, L. and Touraine, R. Lasers, dermal fibroblasts and psoriasis (Correspondence), 744

Saiag, P. *see* Roujeau, J.C.

Salisbury, J. *see* Gibson, J.R.

Salisbury, J. *see* Newton, J.A.

Salle, G. *see* Mauduit, G.

Salo, O.P. *see* Turpeinen, M.

Samman, P.D. *see* Rivers, J.K

Sampaio, S.A.P. *see* Roscoe, J T.

Sarkany, I. *see* Tatnall, F.M.

Sawers, R.S., Randall, V.A. and Ebling, F.J.G. Control of hidradenitis suppurativa in women using combined antiandrogen (cyproterone acetate) and oestrogen therapy, 269

Saxe, N. *see* Hardwick, N.

Schaefer, H. Percutaneous absorption of topically-applied drugs in relation to their toxicity, Suppl. 31, 71

Schalwijk, J. *see* Lammers, A.M.

Schechter, N.M. *see* Lavker, R.M.

Scher, R.K. Acro-osteolyses and the nail unit (Correspondence), 638

Scheynius, A., Johansson, C. and van der Meide., P.H. *In vivo* induction of Ia antigens on rat keratinocytes by $\gamma$-interferon, 543

Schöpf, E. *see* Niedner, R.

Schott, A.M. *see* Mauduit, G.

Schutgens, R.H.B. *see* Cormane, R.H.

Scott, R.C., Thompson, M.A., Ward, R.J., Ramsey, J. and Rhodes, C. *In vitro* absorption of 1-chloro 2,4-dinitrobenzene (DNCB) through human, hooded rat and mouse epidermis, Suppl. 31, 47

Secchi., J. *see* Daniel, J.Y.

Serrano, S. *see* Pedragosa, R.

Selwood, T.S. *see* Marks, R.

Shaw, S. *see* Young, E.

Shaw, S., Wilkinson, J.D., Beck, M., Gobel, C. and Chow, L. Micro-computer-based system for contact dermatitis (Poster summary), Suppl. 30, 36

Sheehan-Dare, R.A., Cotterill, J.A. and Simmons, A.V. Oesophageal lichen planus, 729

Shehade, S.A. and Foulds, I.S. Granulomatous cheilitis and a positive Kveim test, 619

Shelley, W.B. and Burmeister, V. Demonstration of a unique viral structure: the molluscum viral colony sac, 557

Shimao, S. *see* Nakayama, H.

Shipley, M.E. *see* Dowd, P.M.

Shroot, B. *see* Bouclier, M.

Shuster, S. *see* Paramsothy, Y.

Shuttleworth, D., Graham-Brown, R.A.C. and Campbell, A.C. The autoimmune background in lichen planus, 199

Shuttleworth, D. *see* Lister, D.M.

Siddiqui, A.H. *see* Cormane, R.H.

Sillevis Smitt, J.H. and Bakkers, E.J.M. IgA isotypes in linear juvenile IgA dermatosis (Case report), 590

Sillevis Smitt, J.H. *see* Hulsmans, R.F.H.J.

Sillevis Smitt, J.H. *see* Westerhof, W

Simmons, A.V. *see* Sheehan-Dare, R.A.

Simpson, J.M. *see* White, S.I.

Skerrow, C.J. The electron microscope and the antibody. trends in the diagnosis of epidermolysis bullosa, 123

Slater, D.N. and Reilly, G. Fine needle aspiration cytology in dermatology: a clinicopathological appraisal, 317

Slater, D.N. *see* Bleehen, S.S

Slater, D. *see* Kesseler, M.E.

Slater, D. *see* McGrath, H.

Smith, A. *see* Mitchell, D.M.

Smith, I.W. *see* Rüdlinger, R

Smith, N.P. *see* Logan, R.A.

Smith, N.P. *see* Rivers, J.K.

Smith, N.P. *see* Whittaker, S.

Sneddon, I.B. Persistent phototoxicity after benoxaprofen (Correspondence), 515

Sorg, C. *see* Belter, S.V.

Spencer, R.C. *see* Maloney, C

Spittle, M.F. *see* Logan, R.A.

Spittle, M.F. *see* Rivers, J.K.

Spry, C.J.F. *see* Whittaker, S.

Sridharan Iyer, K.S. *see* Puliyel, J.M.

Stahl, K.W. and Kuhne, F.W. Recent findings limiting immunological and photosensitizing hazards of the tetrachlorodecaoxygen anion complex (TCDO) in wound healing promotion, Suppl. 31, 142

Stanford, C.F. *see* Podmore, P

Stapleton, P.J *see* Elwood, J.M.

Stolk, L.M.L., Plasma, A. and Cormane, R.H. Comparison of the *in vitro* dissolution rates of five oral preparations of 8-methoxypsoralen (Summary), 583

Suya, H. *see* Kato, N.

Swanson, N.A., Zachary, C B. and Grekin, R C. Modes of spread of basal cell carcinoma (Summary), Suppl. 30, 23

Swibelius, B.G. *see* Melin, L.

Tagami, H. and Iwatsuki, K. Elevated serum amylase in toxic epidermal necrolysis (Correspondence), 250

Tagami, H. *see* Kato, T.

Tagami, H. *see* Takematsu, H.

Tahan, S.R. *see* Horan, R.F.

Taieb, A., Plantin, P., du Pasquier, P., Guillet, G. and Maleville, J. Gianotti-Crosti syndrome: a study of 26 cases, 49

Takematsu, H., Terui, T., Torinuki, W. and Tagami, H Incontinentia pigmenti: eosinophilic chemotactic activity of the crusted scales in the vesiculobullous stage, 61

Tanner, S. *see* Lister, D.M.

Tatnall, F.M. and Wilson Jones, E. Giant solitary trichoepitheliomas located in the perianal area: a report of three cases, 91

Tatnall, F.M., Dodd, H.J. and Sarkany, I. Elevated serum amylase in toxic epidermal necrolysis (Correspondence reply), 250

Taylor, J.R. *see* Kato, N.

Tea, K. *see* Daniel, J.Y.

Temple, A. *see* Fenton, D.A.

Terui, T. *see* Kato, T.

Terui, T *see* Takematsu, H.

Testa, H.J. *see* Mitchell, D.M.

Thivolet, J. *see* Chardonnet, Y.

Thivolet, J. *see* Mauduit, G.

Thomas, S.E. and Bittiner, S.B. Prognostic factors in allergic contact dermatitis (Summary), Suppl. 30, 25

Thomas, S.E. and Bleehen, S.S. Granulomatous cheilitis (Case report), Suppl. 30, 78

Thomas, S.E and Church, R.E. Cutaneous Crohn's disease (Case report), Suppl. 30, 79

Thomas, S.E. and Church, R.E. Cutaneous sarcoidosis (Case report), Suppl. 30, 75

Thomas, S.E. and Church, R.E Discoid lupus erythematosus (Case report), Suppl. 30, 54

Thomas, S.E. and Church, R.E. Lichen amyloidosis (Case report), Suppl. 30, 59

Thomas, S.E. and Church, R.E. Parasitophobia and linear IgA disease (Case report), Suppl. 30, 83

Thomas, S.E. and Harrington, C.I. Sarcoidois (Case report), Suppl. 30, 76

Thomas, S.E., Church, R.E. and Davies-Jones, G.A.B. Livedo reticularis and cerebro-vascular lesions (Case report), Suppl. 30, 53

Thomas, S.E., Church, R.E. and Holdsworth, C.D. Crohn's disease (Case report), Suppl. 30, 80

Thomas, S.E., Mahood, J. and Church, R.E. Tricho-rhino-phalangeal syndrome (Case report), Suppl. 30, 45

Thompson, M.A. *see* Scott, R.C.

Tidman, M.J. and MacDonald, D.M. Cutaneous endometriosis: a histopathological study (Poster summary), Suppl. 30, 38

Tidman, M.J. *see* Heagerty, A.H.M.

Tokuda, Y. *see* Gondo, A.

Tomlinson, S. *see* Tucker, W.F.G.

Topert, M. *see* Ekerdt, R.

Torinuki, W. *see* Takematsu, H.

Touraine, R. *see* Roujeau, J.C.

Touraine, R. *see* Saiag, P.

Tsutsui, M. *see* Katayama, H.

Tucker, W.F.G. and Bleehen, S.S. Benign familial pemphigus (Hailey-Hailey disease) (Case report), Suppl. 30, 83

Tucker, W.F.G. and Bleehen, S.S. Cutis marmorata telangiectatica congenita (Case report), Suppl. 30, 50

Tucker, W.F.G. and Bleehen, S.S. Granuloma annulare, Suppl. 30, 82

Tucker, W.F.G. and Church, R.E. Atrophic morphoea (Case report), Suppl. 30, 81

Tucker, W.F.G. and Harrington, C.I. Erythrokeratoderma variabilis (Mendes da Costa's syndrome) (Case report), Suppl. 30, 67, 68

Tucker, W.F.G. and Harrington, C.I. Gorlin's syndrome (Case report), Suppl. 30, 95

Tucker, W.F.G. and Harrington, C.I. Klippel-Trenaunay-Parkes-Weber syndrome (Case report), Suppl. 30, 49

Tucker, W.F.G. and Harrington, C.I. Leiomyoma cutis (Case report), Suppl. 30, 93

Tucker, W.F.G. and Harrington, C.I. Systemic mastocytosis (Case reports), Suppl. 30, 62, 64

Tucker, W.F.G., MacNeil, S., Dawson, R.A., Tomlinson, S. and Bleehen, S.S. Increased calmodulin levels in psoriasis: cause or effect? (Poster summary), Suppl. 30, 30

Tucker, W.F.G., Wood, M.L., Bittiner, S.B., Bleehen, S.S. and Church, R.E. Adverse risk factors in atopic eczema: clues from natal interpretation (Poster summary), Suppl. 30, 31

Turbitt, M.L. and MacKie, R.M. An assessment of the diagnostic value of the monoclonal antibodies Leu 8, OKT9, OKT10 and K167 in cutaneous lymphocytic infiltrates, 151

Turpeinen, M., Salo, O.P. and Leisti, S. Effect of percutaneous absorption of hydrocortisone on adrenocortical responsiveness in infants with severe skin disease, 475

Tuyp, E.J. *see* Cox, N.H.

Ulrich, K. *see* Wantzin, G.R.L.

Van Cutsem, J. *see* del Palacio Hernanz, A.

Van de Kerkhof, P.C.M., *see* Lammers, A.M.

Van de Kerkhof, P.C.M., van Huystee, B.E.W L., van Haelst, U.J.G.M. and Bogman, M.J.J.T. Poikiloderma vasculare atrophicans granulomatosa (Correspondence), 383

Van der Linden, M.M.D. Cat scratch disease (Case report), 592

Van der Linden, M.M.D., Hulsmans, R.F.H.J. and Jageneau, A.H.M. The effect of ketanserin on Raynaud's phenomenon in scleroderma patients. A double-blind placebo-controlled study (Summary), 583

Van der Meide, P.H. *see* Scheynius, A.

Van der Schaar, W.W. Relationship between skin diseases and behaviour variables (Summary), 586

Van Genderen, J. *see* Mol, M.A.E.

Van Haelst, U.J.G.M. *see* van de Kerkhof, P.C.M.

Van Huystee, B.E.W.L. *see* van de Kerkhof, P.C.M.

Van Schooneveld, M.J. *see* Hulsmans, R.F.H.J.

Venning, V.A. and Ryan, T.J. Subcorneal pustular dermatosis followed by pyoderma gangrenosum (Correspondence), 117

Venning, V.A., Frith, P.A., Bron, A.J., Millard, P.R. and Wojnarowska, F. Ocular and oral involvement in bullous and cicatricial pemphigoid: a clinical and immunopathological study (Summary), Suppl. 30, 19

Verret, J.L. *see* Burge, S.M.

Viac, J. *see* Chardonnet, Y.

Vidal, J. *see* Pendragosa, R.

Vitale, G.I., Quatrale, R.P., Giles, P.J. and Birnbaum, J.E. Electrical field stimulation of isolated primate sweat glands, 39

Walcyk, P.J. and Elpern, D.J. Brachioradial pruritus: a tropical dermopathy, 177

Walker, A. Anetoderma of Jadassohn (Case report), Suppl. 30, 102

Walker, A.E. Lupus erythematosus profundus (Case report), Suppl. 30, 55

Walker, A.E. *see* Messenger, A.G.

Walker, D.J. *see* Green, C.A.

Walker, E.M. *see* Ferguson, J.

Walker, M.M., Griffiths, C.E.M., Leonard, J.N., Powles, A.V., Weber, J., Harris, W. and Clayton, R.J. Dermatological conditions associated with HTLV-III virus infection (Summary), Suppl. 30, 16

Wallington, T.B. *see* Warin, R.P.

Wallis, P. *see* Phillips, T.J.

Wallis, P.J. *see* Phillips, T.J.

Wantzin, G.R.L., Lindhardt, B.Ø., Weismann, K. and Ulrich, K. Acute HTLV III infection associated with exanthema, diagnosed by seroconversion, 601

Ward, J.D. *see* Wood, M.L.

Ward, R.J. *see* Scott, R.C.

Warin, R.P., Cunliffe, W.J., Greaves, M.W. and Wallington, T.B. Recurrent angioedema: familial and oestrogen-induced, 731

Warnes, T.W. *see* Mitchell, D.M.

WEBER, J. *see* WALKER, M.M.
WEISMANN, K. *see* WANTZIN, G.R.L.
WEPIERRE, J. *see* COURTHEOUX, S.
WESTERHOF, W. Dermatoscopy (Summary), 585
WESTERHOF, W., FLEURY, P. and SILLEVIS SMITT, J.H. Phakomatosis pigmentovascularis type IVb (Case report), 589
WESTERHOF, W. *see* CORMANE, R.H.
WESTERHOF, W. *see* HULSMANS, R.F.H.J.
WHITE, I.R. *see* ENGLISH, J.S.C.
WHITE, J.E. *see* FAIRRIS, G.M.
WHIRE, S.I., FRIEDMANN, P.S., MOSS, C. and SIMPSON, J.M. The effect of altering area of application and dose per unit area on sensitization by DNCB, 663
WHITE, S.I., PUTTICK, L. and MARKS, J.M. Low-dose etretinate in the maintenance of remission of palmoplantar pustular psoriasis, 577
WHITTAKER, S., SMITH, N.P. and RUSSELL JONES, R. Solitary morphoea profunda (Poster summary), Suppl. 30, 37
WHITTAKER, S., SPRY, C.J.F. and RUSSELL JONES, R. Lymphomatoid papulosis: a rare manifestation of the hypereosinophilic syndrome (Summary), Suppl. 30, 18
WIGHTMAN, J. *see* EVANS, C.D.
WILKINSON, J.D. *see* FENTON, D.A.
WILKINSON, J.D. *see* SHAW, S.
WILKINSON, J.D. *see* WILLIS, C.M.
WILKINSON, J.D. *see* YOUNG, E.
WILLEMZE, R. Expression of T6 antigen on the epidermal keratinocytes in various dermatoses (Correspondence reply), 513
WILLIS, C.M., YOUNG, E., BRANDON, D.R. and WILKINSON, J.D. Immunopathological and ultrastructural findings in human allergic contact and irritant dermatitis, 305
WILSON JONES, E. *see* TATNALL, F.M.
WISE, P. *see* MUHLEMANN, M.F.
WISHART, J.M. Bazex paraneoplastic acrokeratosis, 595
WOJNAROWSKA, F. *see* BARTH, J.H.
WOJNAROWSKA, F. *see* VENNING, V.A.
WOLTHUIS, O.L. *see* MOL, M.A.E.
WOOD, M.L. and CHURCH, R.E. Angio-leiomyoma (Case report), Suppl. 30, 94
WOOD, M.L. and CHURCH, R.E. Protein losing enteropathy due to SLE (Case report), Suppl. 30, 58
WOOD, M.L. and HARRINGTON, C.I. Bullous eruption on haemodialysis (pseudoporphyria) (Case report), Suppl. 30, 88
WOOD, M.L. and HARRINGTON, C.I. Dominant dystrophic epidermolysis bullosa (Pasini variant) (Case report), Suppl. 30, 86
WOOD, M.L. and HARRINGTON, C.I. Lymphomatoid granulomatosis (Case report), Suppl. 30, 72
WOOD, M.L. and HARRINGTON, C.I. Recessive dystrophic epidermolysis bullosa (Case report), Suppl. 30, 86
WOOD, M.L., BLEEHEN, S.S. and WARD, J.D. Diabetes, necrobiosis lipoidica and granuloma annulare (Case report), Suppl. 30, 81
WOOD, M.L., CHURCH, R.E. and KESSELER, M.E. Two siblings with epidermolysis bullosa (Case report), Suppl. 30, 85
WOOD, M.L. *see* BLEEHEN, S.S.
WOOD, M.L. *see* MALONEY, C.
WOOD, M.L. *see* TUCKER, W.F.G.

YAMAMURA, T. *see* OKADA, N.
YOSHIKAWA, K. *see* HASHIMOTO, K.
YOSHIKAWA, K. *see* MORIMOTO, S.
YOUNG, E., PEPPER, M., EVERARD, J., SHAW, S. and WILKINSON, J.D. Attitudes to additives—a preliminary report (Poster summary), Suppl. 30, 36
YOUNG, E. *see* WILLIS, C.M.

ZACHARY, C.B. *see* SWANSON, N.A.
ZESCH, A. Short- and long-term risks of topical drugs, Suppl. 31, 63

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# Dermatological Research

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Volume 275 Number 4 1983

**T. B. Fitzpatrick**: In memoriam Professor Makoto Seiji 211

Original Contributions

**R. Ogura, T. Ueda, S. Kumano, T. Sakata, E. J. Zöllner, R. K. Zahn**: Microdisc Electrophoretic Study of Deoxyribonucleases in Cow Snout Epidermis 213

**P. El Baze, G. Milano, P. Verrando, N. Renée, J. P. Ortonne**: Polyamine Levels in Normal Human Skin. A Comparative Study of Pure Epidermis, Pure Dermis, and Suction Blister Fluid 218

**B. Staberg**: Skin Vessel Leakage of Plasma Proteins After PUVA Therapy 222

**A. Langner, T. P. Chorzelski, M. Fraczykowska, S. Jabłońska, J. Szymańczyk**: Is Chemotactic Activity of Polymorphonuclear Leukocytes Increased in Psoriasis? 226

**C. Papageorgiou, J.-P. Corbet, F. Menezes-Brandao, M. Pecegueiro, C. Benezra**: Allergic Contact Dermatitis to Garlic (*Allium sativum L.*). Identification of the Allergens. The Role of Mono-, Di-, and Trisulfides Present in Garlic. A Comparative Study in Man and Animal (Guinea-Pig) 229

**W. Kraus, P. Schramm, N. Hoede**: First Experiences with a High-Resolution Ultrasonic Scanner in the Diagnosis of Malignant Melanomas 236

**P. J. Dykes, D. L. Williams, R. Marks**: Non Identity of Non Ionic Detergent Soluble (NIDS) Protein and Stratum Corneum Autoantigens 239

**D. A. W. Bucks, H. I. Maibach, E. Menczel, R. C. Wester**: Percutaneous Penetration of Hydrocortisone in Humans Following Skin Delipidization by 1,1,1-Trichlorethane 242

**J. Faergemann, R. Aly, H. I. Maibach**: Adherence of *Pityrosporum orbiculare* to Human Stratum Corneum Cells 246

**A. A. Hartmann**: Effect of Occlusion on Resident Flora, Skin-Moisture and Skin-pH 251

Short Communications

**C. Kuehnl-Petzoldt, H. Wiebelt, H. Berger, G. Wagner**: A Proposal for Staging Malignant Melanoma 255

**F. Ott, J. M. Geiger**: Therapeutic Effect of Arotinoid Ro 13-6298 in Psoriasis 257

**J. R. Jensen, M. El'Ramley, T. Herlin, K. Kragballe, K. Thestrup-Pedersen**: Etretinate Therapy and Immune Reactivity 259

**J. Karvonen, J. Ilonen**: The Effect of PUVA or UVB Treatment on the PHA Responsiveness of Peripheral Lymphocytes in vivo 261

Abstract

Arbeitsgemeinschaft Dermatologischer Forschung (ADF) *in Cooperation with „Deutsche Dermatologische Gesellschaft"* X. Annual Meeting, November 19–21, 1982 Münster, FRG 263

Announcement 286

Indexed in Current Contents

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| gram(s) | g | minute(s) | min | molar | mol/l |
| kilogram(s) | kg | centimetre(s) | cm | millilitre(s) | ml |
| milligram(s) ($10^{-3}$g) | mg | second(s) | s | gravitational acceleration | *g* |
| microgram(s) ($10^{-6}$g) | μg | cubic millimetre(s) | $mm^3$ | micrometre(s) | μm |
| nanogram(s) ($10^{-9}$g) | ng | millimetre(s) | mm | per cent | % |
| picogram(s) ($10^{-12}$g) | pg | millicurie(s) | mCi | isotopic mass number placed as | $^{131}$I |
| hours(s) | h | milliequivalent | mmol | | |

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British Journal of Dermatology, Volume 115, Number 6, December 1986

CONTENTS PAGE

## Comment

Nomenclature of non-neoplastic vulval conditions 647
C.M.RIDLEY

## Clinical and Laboratory Investigations

Spontaneous remission of solar keratoses: the case for conservative management 649
R.MARKS, P.FOLEY, G.GOODMAN, BRIDGET H.HAGE AND T.S.SELWOOD
Pulmonary function in patients on long-term, low-dose methotrexate 657
T.J.PHILLIPS, P.J.WALLIS, D.H.JONES AND H.BAKER
The effect of altering area of application and dose per unit area on sensitization by DNCB 663
S.I.WHITE, P.S.FRIEDMANN, CELIA MOSS AND JUDY M.SIMPSON
Langerhans cells in human warts 669
YVETTE CHARDONNET, JACQUELINE VIAC AND J.THIVOLET
The effect of thalidomide on arachidonic acid metabolism in human polymorphonuclear leukocytes and platelets 677
P.D L.MAURICE, A.S.J.BARKLEY AND B.R.ALLEN
Human papillomavirus infections in a group of renal transplant recipients 681
R.RUDLINGER, I.W SMITH, M.H.BUNNEY AND J.A.A.HUNTER

## Case Reports

Erythroderma associated with mixed lymphocyte-endothelial cell interaction and *Staphylococcus aureus* infection 693
MADALENE C.Y.HENG, S.G.KLOSS AND D.G.CHASE
Longitudinal melanonychia with spreading pigmentation in Laugier-Hunziker syndrome: a report of two cases 707
R.BARAN AND H.BARRIÈRE
Congenital smooth muscle hamartoma: a case report 711
BRENDA J.BERBERIAN AND J.W.BURNETT
Perineural xanthoma 715
H.NAKAYAMA, M.MIHARA AND S.SHIMAO
Release of prostaglandin $D_2$ and histamine in a case of localized heat urticaria, and effect of treatments 721
O.KORO, J.S.DOVER, D.M.FRANCIS, A.KOBZA BLACK, R.W.KELLY, R M.BARR AND M.W.GREAVES
Oesophageal lichen planus 729
R.A.SHEEHAN-DARE, J.A.COTTERILL AND A.V.SIMMONS
Recurrent angioedema: familial and oestrogen-induced 731
R.P.WARIN, W.J.CUNLIFFE, M.W.GREAVES AND T.B.WALLINGTON
Acropustulosis of infancy 735
JULIA A.NEWTON, JENNIFER SALISBURY, A.MARSDEN AND D.H.MCGIBBON

## Obituary

Claude Howard Whittle 741

## Correspondence

Lichen sclerosus et atrophicus in non-identical female twins 743
N.H.COX, J.N.S.MITCHELL AND W.N.MORLEY
Gamma-glutamyl transpeptidase as a marker for distinguishing malignant epithelial tumours 743
S.T.HUSSAIN
Influence of containers on the quantities of topical preparations used 743
C.D.EVANS AND J.WIGHTMAN
Lasers, dermal fibroblasts and psoriasis 744
P.SAIAG, B.COULOMB, C.M.E.ROWLAND-PAYNE, L.DUBERTRET AND R.TOURAINE
Cyclosporin A treatment of severe psoriasis 745
JANET M.MARKS

**Book Reviews** 747

**News and Notices** 750

**Index** 751